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THE BLACK-BUNDLE DISEASE OF CORN

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INTRODUCTION

As the symptoms produced by organisms known to be parasites of corn became more clearly differentiated to the writers, there remained several groups of disease symptoms for which the explanations were meager or lacking. This paper deals with one of these groups of symptoms in a general way and specifically assigns an organism, not hitherto shown to be a parasite of corn, as one of the important causes.

SYMPTOMS

Occasionally stripelike lesions are found in the leaves of young, infected corn plants (Pl. 6, A). Usually, however, black-bundle disease symptoms do not become evident during the first half of the growing season. During ear development various symptoms may appear as: Leaf and stalk color manifestations (Pl. 1), barren stalks (Pl. 2 A, B), nubbin ears (Pl. 2 B), prolific stalks (Pl. 2 C), or excessive suckering (Pl. 4 A, B). Such stalks usually show blackened fibrovascular bundles (Pl. 1).

In every field of dent, flint, and sweet corn (*Zea mays indentata*, *Z. mays indurata*, and *Z. mays saccharata*) under observation by the writers it has been noted that a number of plants have become red or purple on reaching the dough stage. The red coloration appears first at or near the midvein of the topmost leaf and progresses downward on the plant, affecting several leaves before progress on the stalk commences. In extreme cases the stalks and all the leaves become reddish purple, but all gradations between the initial appearance of the red color and this extreme condition may be the final color symptoms. Plants having any gradation of this reddening or purpling are designated in this paper as purple-leaf plants.

This type of reddening does not conform to any of those described by Emerson (*14*) [2] who says:

> It is of interest to recall in this connection that plant colors of maize—brown no less than the red-purple series—develop first in the older parts where growth first ceases, such as the lower sheaths and the upper parts of the internodes of the culm.

However, a type of reddening, indistinguishable from the one encountered in commercial fields, sometimes occurs in selfed lines of dent corn. Inoculation of open fertilized dent corn with a particular organism, as will be shown later in this paper, increases the number of purple or red plants.

This disease is characterized also at this stage of development of the corn plant by high percentages of barren stalks and stalks producing

[1] Accepted for publication Nov. 24, 1923. The investigations here reported were conducted in cooperation with the Funk Bros. Seed Co., Bloomington, Ill., and the Wisconsin and Illinois Agricultural Experiment Stations.

[2] Reference is made by number (italic) to "Literature cited," p. 203.

nubbin ears only (Pl. 2, A, B). Often imperfectly developed ears are observed at a number of nodes on a stalk and more frequently multiple-ear production occurs at one node (Pl. 2, C), showing futile attempts at prolificacy. These manifestations, and large succulent stalks should be included, are the readily apparent symptoms associated with the black-bundle disease of corn and can be noted in any commercial field. When plants having any of these symptoms are cut open, blackened vascular bundles can nearly always be found in the nodes and internodes near the base and sometimes throughout the stalk (Pl. 1 and Pl. 3, B). Occasionally the fundamental tissue outside the blackened vascular tissue becomes browned or blackened, but usually in only one internode of a stalk.

However, the number of plants having these symptoms (excluding the black-bundle symptom) represents only a small part of the total number of plants affected by the disease. Plants, including the ears, may appear outwardly healthy and yet be infected from the crown to the tassel and every kernel on fine looking ears may have the organism internally. In these cases the presence of the black vascular bundles within the stalks is the most distinguishing symptom. The ears may be diagnosed by plating the kernels or by germination in conjunction with microscopic examination.

Apparently good seed ears selected from apparently normal stalks and producing 100 per cent of strong plants on the germinator may produce high percentages of purple plants in the field. No microscopic examination of seedlings on the germinator was made to determine infections. In these cases plant growth records during the first half of the season usually indicated strong rows. Table I shows in detail the record of an ear of the kind described and compares it with the records of four other ears. Among other things, it is an illustration of the statement that no appreciable indications of this group of symptoms are to be found during the first half of the growing season.

TABLE I.—*Field performance of an infected ear row (row 3) compared with adjacent uninfected ear rows; Funk Ninety-Day corn planted May 24, 1919, in brown silt loam soil of medium fertility, at Bloomington, Ill.*

Notations at different dates.	Condition of seed and accession numbers of seed ears.				
	Uninfected.		Infected.	Uninfected.	
	Row 1, No. 5794.	Row, 2, No. 5831.	Row 3, No. 5824.	Row 4, No. 5887.	Row 5, No. 5897.
Stand and early vigor, June 13:					
Total stand	146	123	136	143	138
Per cent stand	97	82	91	95	92
Number strong plants	107	61	96	108	71
Number weak plants	6	11	12	7	5
Number blighted plants	1	3	0	1	1
General condition	Good.	Medium.	Good.	Good.	Good.
Condition, July 11 and 12:					
Color of foliage	Dark green.	Dark green.	Dark green.	Dark green.	Dark green.
Uniformity of growth	Uniform.	Variable.	Uniform.	Uniform.	Uniform.
Number leaning plants	3	3	2	22	16
Condition, Aug. 6:					
Number plants with wilting or dead leaves	3	15	5	3	27
Preharvest data, Sept. 11:					
Number good ears	131	99	40	132	117
Number mid-sized ears	1	2	10	1	6
Number nubbins	2	9	18	5	2
Number barren plants	6	22	54	2	9
Number smutted plants	6	7	7	3	2
Number prematurely dead plants	0	0	7	0	2
Percentage of purple plants	0	0	85	0	0
Acre yield in bushels	90	57.8	37.4	69.7	62

The seed ears referred to in Table I were the first generation crosses (artificially pollinated) of different strains of Funk Ninety Day, and when tested on the germinator, 30 kernels from each, showed 100 per cent vigorous seedlings and no evidence of rotting. Hence, it was thought that all were healthy. However, on September 11, row 3 (seed-ear 5824) was found to be uniformly diseased. As shown in Table I, there is a decrease in number of good ears and an increase in number of nubbins, barren stalks, and stalks with purple leaves, in comparison with adjacent rows on each side. Numerous stalks in row 3, when cut open at time of harvest, showed the characteristic blackened bundles. Usually in the latitude of central Illinois, the time of appearance of any easily recognized symptom of this disease is about the third week of August. At that time the first purple leaves usually appear, and barren and prolific stalks can be identified.

Symptoms as described and certain related observations have been noted for a number of years. For example, in the fall of 1917, an ear row of Funk Ninety-Day corn in a breeding plat showed certain outstanding characteristics. The plat was located on clover sod that had not grown corn for three years. The soil was uniform and of medium fertility. The ear from which the row in question was grown was of fine appearance in every respect and had shown strong germination on the germinator with no evidence of rotting. Hence it had been selected as a choice ear for breeding stock. During the early part of the season the plants showed no signs of disease. Attention was first directed to this row early in September following a light frost. All of the plants in this row were slightly injured by the frost, while the plants in adjacent rows showed no injury whatever. The row also contained an unusually large proportion of purpled plants and an abnormally large percentage of barren stalks. Further, on cutting open the stalks, it was found that most of the barren stalks and those with nubbins as well as some with good ears contained abundant blackened bundles. The row yielded only about one-half as much as the best adjoining rows. Seed ears for use in an experiment the following year (1918) were selected from this row, both from plants showing blackened bundles and from those which apparently were healthy. Eleven ears were thus selected, two from the apparently healthy plants and nine from the diseased ones. These ears were tested on the germinator in the usual way and all showed 100 per cent of strong germination, with no evidence of rotting. In 1918, these 11 ears were planted in ear rows along with 6 other ear rows from apparently healthy ears as controls. Unfortunately, the proportions of plants showing purple-stalk and black-bundle symptoms were not noted in 1918, but careful yield data were taken and computed to acre yields on a uniform moisture basis. The nine diseased rows produced an average of only 53.1 bushels per acre compared with 77.8 and 72.8 bushels per acre from the rows from the two specially selected ears and from the six control rows, respectively.

Certain earlier manifestations in the development of corn plants, such as abnormally fast growth in height or diameter, indicate that such plants later on are likely to produce symptoms of purple-stalk and black-bundle diseases. Some evidence that abnormally fast growth in height is an indication of disease was brought out from data obtained in 1919. Three hundred and twenty-seven plants were measured for height 36 days after planting and were grouped in quartiles according to height,

each quartile representing approximately one-fourth of the population. In order to determine the relation of height to production, the individual production of each plant was recorded in grams. The results are summarized in Table II and shown graphically in figure 1.

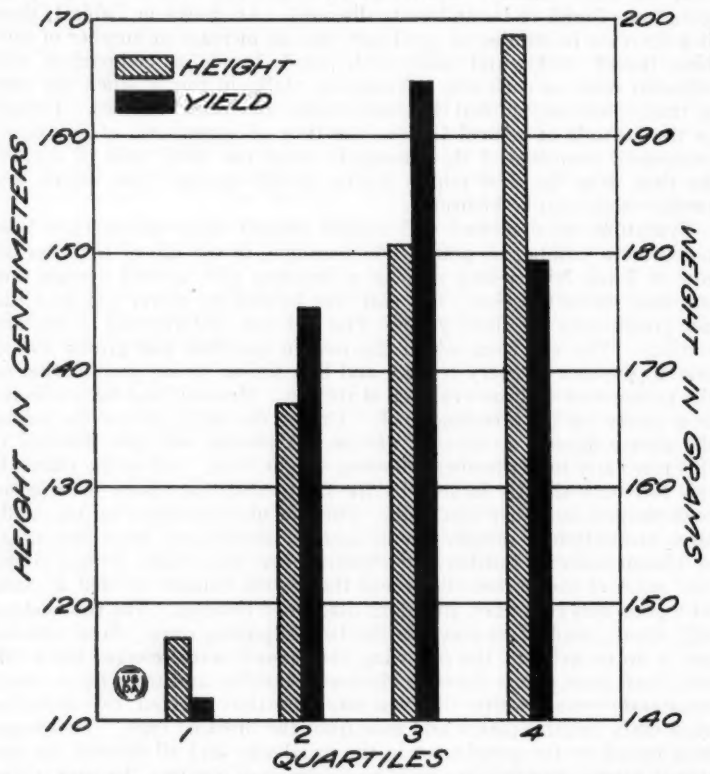

FIG. 1.—Graph showing average height of Yellow Dent corn plants, grouped in quartiles according to height, and average yield of plants in each quartile, respectively. Bloomington, Ill., 1919.

TABLE II.—*Data showing average height of 327 plants of Yellow Dent corn, grouped in quartiles according to height, 36 days after planting, and average yield of plants in each quartile, respectively, grown on brown silt loam soil of high fertility near Bloomington, Ill., 1919 (see also fig. 1)*

Quartile.	Number of plants.	Mean plant height.	Mean plant yield.	Increase in height (per cent).	Increase in production (per cent).
		Cm.	*Gm.*		
1.....	82	116.7	142.1	.....	
2.....	82	137.1	175.6	Second quartile, 17.5...	Over first, 23.6.
3.....	82	150.6	194.4	Third quartile, 9.8.....	Over second, 10.7.
4.....	81	169.0	179.7	Fourth quartile, 12.2...	Over third, —7.6.

There is a direct relation between height of plant and production per plant except in quartile four which is made up of the plants of greatest height. This quartile contained a number of plants which grew abnormally fast. Also a higher percentage of barrenness was noted in this quartile. Briefly stated, in considering, during the first half of the growing season, a number of corn plants having the usual percentage of black-bundle disease, plant yield increases with plant height up to a certain point, and then decreases.

HISTORY

While certain of the symptoms of this group, especially barrenness, has been extensively noted by various investigators, no comprehensive, detailed analysis or investigation of the causes of these abnormalities as a group has been made. Hunt (*24, p. 151*) says:

> A varying percentage of the stalks of the field are barren—do not bear any ears. The percentage of barren stalks on a given soil varies with the thickness of planting and the season. Barrenness does not seem to be a variety characteristic. It seems to be largely the result of environment. If it were an hereditary characteristic the fact that the stalks are barren would tend to eliminate them.

Burtt-Davy (*6, p. 87–90*) in considering barrenness as an hereditary character says that if barren stems were absolutely barren natural elimination would result, but the fact that they produce tassels and pollen lends color to the view that they may tend to reproduce their kind. He also mentions that cases are reported from America of 60 per cent of barren stems in a crop of maize. Blackwell (*2, p. 20*) in reporting the work of Hutchinson at South Carolina in using pollen from barren stalks to determine its effect on the amount of barrenness in the progeny, decided at first that it must be a Mendelian character and inherited in the ratio of 1 to 3; but later results did not bear out that conclusion. He adds that it has also been noted that there is or appears to be a correlation between barrenness and certain other characters, such as color, size of plant, shape of plant, length of life, and possibly others.

Pammel, King, and Seal, 1916 (*37*) described a cornstalk disease in Iowa due to *Gibberella saubinetii*. The stalks often were barren or had small ears. The small dwarf shoots in the axils of the leaves were frequently decomposed and the stalks were abortive. Hoffer and Holbert, 1918 (*19*) state that species of Gibberella, Fusarium, Verticillium, Rhizopus, and Pseudomonas are, in a great measure, responsible for missing hills, slow-growing stalks, barren stalks, down stalks, and early blighting of corn plants. They also say (*20, p. 2, 11*) that barren stalks and stalks bearing nubbins only seem to be correlated with certain pathologic conditions in the plants, * * * that the rate of seedling development usually referred to as "vitality" is not a criterion for assuming freedom from infection of the seed by bacteria and species of Fusarium. The rate of seedling development on the germinator is not indicative of the yield possibilities of that seed ear. Selby, 1918 (*46*), says that rootrot shows in corn not only in weakening the roots, making it easy to blow over, but also in causing dwarfing of growth, premature dying of the top, and barrenness of stalks attacked. The diseased plants may be purplish-colored, dwarfed or stunted, and unproductive. Russell, 1919, (*42*) reports more barren stalks in dry years

such as 1913 and 1914. His observations during one of these years led him to believe the cause due to lack of plant food. The fact that the fields of corn observed were grown from seed from different sources was a factor possibly as important as lack of plant food. Hunt, 1919, (*23*) believes barren stalks are due to lack of fertility, a disease in the plant, or unfavorable weather conditions at time of pollination. Relative to diseases as the cause, he says:

> Many stalks are pronounced barren when one or sometimes several shoots are found which started but failed to develop. If these shoots are examined they will be found to be infected with a mold which probably arrested the development of the ear.

Trost, 1922, (*49*) presented data which showed that 7.2 per cent of corn plants grown from horny seed and 11.4 per cent from starchy seed of the same strain of corn were barren.

Representative corn growers and breeders of Illinois have related the following observations:

Eugene D. Funk, of Shirley, Ill., writes in a letter of December 11, 1922:

> The notes from our field men, as far back as 1901, show that we had more or less barren stalks in our breeding plats. At that time, however, we only made reference to the absence of ears and were of the opinion that the cause was due solely to heredity. More recent and careful study of the corn plant brought our attention to the color and development of the foliage, as well as the color of the stalk at harvest time, and now we definitely recognize that the purple, barren stalks are largely the result of a disease of the bundles.

Frank I. Mann, Gilman, Ill., writes in a letter of November 26, 1921:

> If we had the purplish stalks and leaves in early times, I do not remember noticing them. For about 20 years, however, we have had many of them. As many of them produce very good ears, sometimes we could not associate this character with barrenness as fully as we do now, but still recognized it as very undesirable. There were a good many of these purple plants in our corn this fall. It seems to be the prevailing cause of complete and partial barrenness in our corn this season.

Harvey J. Sconce, Sidell, Ill., writes in a letter of December 5, 1921:

> You are right in remembering that I had made a statement that in riding through cornfields on a horse or walking I had detected diseased corn plants several feet ahead of me. This was particularly the case again this year as the purple-stalk disease was quite heavy. They were in general all barren, had suckered badly, and every one was easily detected. I early learned to detect these plants as my breeding records date back to 1904 and as early as 1908 to 1910, I made notes on several rows that showed a greater percentage of these plants than other rows.

It is seen from the foregoing reports that both corn growers and investigators have described symptoms, such as barrenness, purple leaves and stalks, and production of nubbin ears. All of these sypmtoms fall into the group described herein and associated with the effects of parasitism by the organism discussed later. As these symptoms are often associated with other causes, the black-bundle symptom is chosen as the most distinctive, hence the common name.

In the early work on corn diseases by one of the authors (J. R. Holbert), the black bundles sometimes found in diseased corn were considered one of the symptoms of the corn root, stalk, and ear rots caused by species of Fusarium. This belief seemed to be shared by other workers. It was noted as early as 1917 that purple leaves and barrenness often were associated with the occurrence of black bundles. In the fall of 1919 the writers gave special attention to an ear row in which these symptoms developed in about 85 per cent of the plants. After examining the roots of some of the plants to a depth of 6 feet with negative results as to the presence of lesions of any importance, it was apparent that the cause

might not be due to species of Fusarium. Numerous isolations from the black bundles found in these plants consistently gave one organism, and pure-culture inoculations with it in the greenhouses at the University of Wisconsin in November, 1919, proved it to be pathogenic on corn. Without doubt this organism was responsible for the symptoms developed in the row in question. Subsequent investigations have shown, however, that symptoms almost identical with these may develop from other causes.

GEOGRAPHICAL DISTRIBUTION

The black-bundle disease of corn has been noted in Connecticut, New York, Ohio, Indiana, Illinois, Wisconsin, Minnesota, Iowa, South Dakota, Kansas, South Carolina, and California. This distribution renders it probable that it occurs wherever corn is grown in the United States.

ECONOMIC IMPORTANCE

The prevalence of this group of symptoms and their effect on productiveness of corn plants is such as to make it important that the causative agents be studied carefully and some effort made to lessen their occurrence. During 1919, 1920, and 1921, surveys of commercial fields near Bloomington, Ill., were made to determine the occurrence and productiveness of corn plants having the purple-leaf-and-stalk symptom. The results are summarized in Table III.

TABLE III.—*Occurrence of purple plants in some commercial fields of Yellow Dent corn near Bloomington, Ill., grown from seed selected from plants which showed no symptoms of the black-bundle disease*

Year.	Number of plants examined.	Purple plants (main stalks.)				
		Total.		Bearing.		Barren.
				Ears.	Nubbins.	
		Number.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1919	666	47	7.1	34.0	12.8	53.2
1920	4,857	351	7.2	27.9	31.9	40.2
1921	2,068	112	5.4	28.0	14.0	58.0

In addition to the preceding detailed observations, the authors have determined by count the percentages of purple-leaf plants occurring in a few scattered commercial corn fields in Indiana, Iowa, and Kansas, and at various places in Illinois. These have varied from a trace to 15 per cent. On the whole, however, it is believed that the data in the preceding table are fairly representative.

During 1920, in ear-row experimental plats of Yellow Dent corn at Bloomington, Ill., observations were made on 27 ear rows to determine the occurrence of purpling in main stalks and suckers. The relative number of purple-leaf plants which were barren or bore ears or nubbins also was noted. The seed ears were from plants that had not shown purpling in 1919. In planting the plats, twenty kernels were planted singly 14 inches apart in rows 42 inches apart. The results are given in Table IV.

TABLE IV.—*Data on the occurrence of purple-leaf symptoms developed in experimental plats of Yellow Dent corn grown in 1920 near Bloomington, Ill., from seed ears selected from plants not showing purple-leaf symptoms, the seed being untreated*

Row No.	Hills, total number.	Suckers.		Plants (main stalks) without purple leaves.	Plants (main stalks) with purple leaves.			
		Total.	Purpled.		Total number.	Bearing.		Barren.
						Ears.	Nubbins.	
1	15	25	2	12	3	2	1	0
2	14	25	3	12	2	2	0	0
3	16	26	2	15	1	1	0	0
4	11	13	1	10	1	1	0	0
5	15	21	0	15	0	0	0	0
6	17	33	4	15	2	1	0	1
7	17	21	0	16	1	0	1	0
8	15	18	7	14	1	1	0	0
9	16	19	0	16	0	0	0	0
10	13	30	1	12	1	1	0	0
11	19	29	1	18	1	0	1	0
12	17	26	4	16	1	1	0	0
13	5	8	0	5	0	0	0	0
14	17	16	2	17	0	0	0	0
15	17	11	0	17	0	0	0	0
16	17	39	0	17	0	0	0	0
17	19	44	9	17	2	1	1	0
18	15	29	3	15	0	0	0	0
19	16	28	2	16	0	0	0	0
20	17	38	6	15	2	0	2	0
21	18	24	3	18	0	0	0	0
22	18	35	6	16	2	1	1	0
23	13	28	1	13	0	0	0	0
24	12	27	0	12	0	0	0	0
25	11	28	6	10	1	1	0	0
26	16	35	1	15	1	1	0	0
27	12	25	2	10	2	1	1	0
Total	408	701	66	384	24	15	8	1

Table IV presents data which, when expressed in percentages, show that 5.9 per cent of the plants had the color symptom that was used as a basis in these data, and the relative percentages of these plants that were barren (4.2), nubbin-producing (33.3), and ear-producing (62.5).

In 1919, ears of Yellow Dent corn were selected from purple plants with a view to determine their field performance in 1920. In planting the plats, 40 kernels were planted singly 14 inches apart in rows 42 inches apart. The results from 23 of these ears are given in Table V.

On the basis of the data presented in Table V, it is found: (1) That 46.6 per cent of main stalks and 43.2 per cent of all suckers in these rows showed the purple-leaf symptoms; (2) that of the plants with the purple-leaf symptom, 55.1 per cent produced ears, 27.5 per cent produced nubbins, and 17.4 per cent were barren; and (3) that the mean yield of plants showing the purple-leaf symptom was 176 gm. per plant as compared with 264.2 gm. per plant from apparently healthy plants in the same ear rows, thus showing 33.4 per cent reduction in yield per plant by those plants having the purple-leaf symptom.

Along with the experiments referred to in Tables IV and V, another experiment was conducted in 1920 to determine if seed treatment with

corrosive sublimate would eliminate the purple-leaf symptoms. In the matter of control the results were almost negative, but the data obtained were important in other ways, and hence they are included at this point.

The ear rows were planted with seed from the same ears as those used for the experiment referred to in Table V, which were selected from purple-leaf plants. The kernels were planted singly 14 inches apart in rows 42 inches apart. This seed was treated by immersion for one-half hour in a 1 : 1000 mercury bichlorid solution. The results are given in Table VI.

TABLE V.—*Data on the occurrence of purple-leaf symptoms and on yield of Yellow Dent corn grown near Bloomington, Ill., 1920, in ear-row experimental plats, from seed ears selected from plants showing purple-leaf symptoms in 1919, the seed used in 1920 being untreated*

Row No.	Number of—			Plants without purple-leaf symptoms.				Plants with purple-leaf symptoms.						
				Number of—; Yield.				Number of—		Yield.		Number producing—		
	Plants.	Suckers.	Purple suckers.	Plants.	Ears.	Total.	Average per plant.	Plants.	Ears.	Total.	Average per plant.	Ears.	Nubbins.	Barren.
						Gm.	*Gm.*			*Gm.*	*Gm.*			
1	22	44	25	11	11	2,761	251	11	10	2,080	189	7	3	1
2	25	31	24	11	11	2,448	223	14	9	2,012	144	5	4	5
3	27	31	10	19	20	4,848	255	8	7	1,402	175	4	3	1
4	31	47	28	7	7	1,944	278	24	19	3,418	142	10	9	5
5	25	38	19	8	8	2,068	259	17	15	2,840	167	8	7	2
6	32	43	11	20	20	5,661	283	12	10	2,268	189	7	3	2
7	26	36	18	8	8	2,071	259	18	16	3,288	183	10	6	2
8	25	26	15	12	12	3,172	264	13	9	1,942	149	6	3	4
9	31	51	13	19	22	5,600	295	12	14	3,055	255	9	2	1
10	29	22	12	17	17	4,766	280	12	12	2,198	183	7	5	0
11	24	30	18	14	14	3,972	284	10	9	2,273	227	8	1	1
12	29	43	25	7	7	2,000	286	22	20	4,044	184	16	4	2
13	32	43	18	18	18	4,519	251	14	14	2,636	188	7	7	0
14	28	30	11	18	18	5,253	292	10	6	1,386	139	4	2	4
15	17	10	8	14	14	4,098	293	3	3	792	264	2	1	0
16	30	40	17	19	19	5,548	292	11	9	2,509	228	9	0	2
17	31	44	7	25	26	7,255	290	6	4	993	166	4	0	2
18	34	26	8	18	18	4,172	232	16	13	3,011	188	7	6	3
19	11	12	3	9	9	1,873	208	2	2	200	100	1	1	0
20	16	9	3	9	9	2,228	248	7	6	1,134	162	4	2	1
21	15	18	11	3	3	672	224	12	7	1,600	133	5	2	5
22	15	15	0	12	12	3,494	291	3	2	214	71	1	1	1
23	14	15	6	6	6	1,426	238	8	6	1,340	168	5	1	2
Total	569	704	304	304	309	81,849	264.2	265	222	46,629	176	146	73	46

Table VI shows that, although treated for one-half hour in a 1:1000 solution of mercury bichlorid, a high percentage (42.7 per cent) of purple plants resulted from planting seed selected from purple plants and that purple plants yielded less (20.6 per cent) than apparently normal plants from the same ears. A comparison of this table with Table V is shown in Table VII.

It will be noted from the summaries given in Table VII that, although no important effect resulted from this treatment, there was a slight decrease in the percentage of plants with the purple-leaf symptom and an increase in the yields of both healthy and diseased plants. In general, however, the results show the desirability of selecting seed ears only from plants not showing the purple-leaf symptom. This statement is

made because the data show that ears selected from such diseased plants produced 46.6 and 42.7 per cent plants with the purple-leaf symptom, respectively, as compared with 5.9 per cent plants with the same symptom in corresponding ear rows from seed selected from apparently healthy plants (Table IV).

TABLE VI.—*Data on the occurrence of the purple-leaf symptom and on yield of Yellow Dent corn grown near Bloomington, Ill., 1920, in ear-row experimental plats from seed ears selected from plants having the purple-leaf symptom in 1919, the seed being treated with 1:1000 mercury bichlorid solution for one-half hour immediately before planting (1920)*

Row No.	Number of—			Plants without purple-leaf symptoms.				Plants with purple-leaf symptoms.						
				Number of—		Yield.		Number of—		Yield.		Number producing—		
	Plants.	Suckers.	Purple suckers.	Plants.	Ears.	Total.	Average per plant.	Plants.	Ears.	Total.	Average per plant.	Ears.	Nubbins.	Barren.
						Gm.	*Gm.*			*Gm.*	*Gm.*			
1	11	17	9	5	5	1,470	294	6	5	762	127	2	3	1
2	14	15	11	7	7	1,856	265	7	5	1,264	181	3	2	2
3	17	30	6	11	11	2,614	238	6	5	1,144	191	4	1	1
4	17	19	12	7	7	1,754	251	10	7	1,454	145	5	2	3
5	16	26	13	3	3	800	267	13	12	2,202	169	6	6	1
6	16	24	8	10	16	2,713	271	6	5	1,166	194	3	2	1
7	16	12	2	12	12	3,086	257	4	4	1,188	297	3	1	0
8	15	23	16	7	7	2,165	309	8	7	2,296	287	7	0	1
9	16	20	13	7	7	2,090	299	9	9	2,372	264	8	1	0
10	15	9	0	12	12	3,372	281	3	3	746	249	3	0	0
11	11	9	4	9	9	2,788	310	2	1	304	152	1	0	1
12	15	17	13	11	11	3,026	275	4	3	810	203	3	0	1
13	16	13	5	7	7	1,484	212	9	8	1,786	198	4	4	1
14	11	14	3	10	10	3,488	349	1	1	290	290	1	0	0
15	6	2	0	5	5	1,612	322	1	1	346	346	1	0	0
16	18	27	6	14	14	4,320	309	4	4	1,122	281	3	1	0
17	16	12	4	12	12	3,170	264	4	4	1,192	298	4	0	0
18	18	14	3	11	11	2,950	268	7	5	1,198	171	3	2	2
19	14	18	15	4	4	940	235	10	9	1,688	169	4	5	1
20	13	8	5	6	6	1,450	242	7	7	1,104	158	2	5	0
21	17	27	12	8	8	2,098	262	9	7	1,730	192	6	1	2
22	15	16	4	11	11	2,980	271	4	4	1,072	268	4	0	0
23	14	24	15	4	4	934	234	10	8	2,032	203	6	2	2
Total	337	396	179	193	199	53,160	275.4	144	124	29,268	218.8	86	38	20

TABLE VII.—*Summary of data given in Table V, where seed was untreated, and Table VI, where seed was treated with mercury bichlorid solution 1:1000 for one-half hour. The seed in both cases was from the same ears selected from purple-leaf plants*

Points of comparison.	Table V, seed untreated	Table VI, seed treated.
Percentage of all plants that showed purple-leaf symptoms	46.6	42.7
Percentage of all suckers that showed purple-leaf symptoms	43.2	45.2
Average yield of apparently healthy plants	264.2	275.4
Average yield of purple-leaf plants	176.0	218.8
Average percentage reduction in yield from purple-leaf plants	33.4	20.6
Percentage of plants with the purple-leaf symptom that produced ears	55.1	59.7
Percentage of plants with the purple-leaf symptom that produced nubbins	27.5	26.4
Percentage of plants with the purple-leaf symptom that were barren	17.4	13.9

During the same year (1920) an experiment was conducted to compare plant yields of corn grown from ears selected in 1919 from purple-leaf plants and corresponding ones from plants not showing this symptom. Twenty-seven ear rows of equal length (25 feet) were planted from each lot of ears. Kernels were planted singly, 14 inches apart in rows 42 inches apart. The results are given in Table VIII.

TABLE VIII.—*Comparative yields of 25-foot ear rows of Yellow Dent corn, grown near Bloomington, Ill., in 1920, from ears selected in 1919 from purple-leaf plants and from plants without the purple-leaf symptom*

Row No.	Seeds selected from purple-leaf plants.		Row No.	Seeds selected from plants without purple-leaf.	
	Number of plants.	Yield.		Number of plants.	Yield.
		Gm.			Gm.
1	11	2,136	9B	15	4,899
2	11	2,300	9C	17	3,460
3	11	2,320	9T	15	4,360
4	16	2,132	18B	16	3,682
5	16	2,692	18C	17	3,650
6	15	3,206	18T	15	4,534
7	12	2,590	27C	18	3,973
8	14	2,968	27T	18	3,917
9	14	3,422	28	15	3,777
10	15	4,658	29	14	4,554
11	12	3,606	30	16	4,162
12	12	2,724	31	11	3,660
13	18	4,146	32	15	3,689
14	14	3,744	33	17	4,144
15	6	1,880	34	17	3,946
16	16	4,424	35	15	3,962
17	14	4,218	36	16	4,086
18	17	3,524	36C	16	5,020
19	11	2,073	36T	15	3,775
20	16	3,362	39	16	4,313
21	15	2,272	39T	14	3,264
22	15	3,708	41	15	3,718
23	14	2,766	41T	15	3,859
24	15	4,084	45C	18	3,945
25	19	5,016	45T	15	3,434
26	16	3,490	50T	15	3,717
27	15	2,413	54C	20	3,405
Average yield per row		3,181±123.0	Average yield per row		3,959±54.3

On the basis of the data given in Table VIII it will be noted that the average yield from the 27 rows from seed of purple plants was 778 ± 134.5 gm, less than the average yield from the corresponding ear rows grown from seed selected from plants without the purple-leaf symptom. This is a 19.7 per cent reduction in yield, and is significant in terms of the probable error involved.

CAUSES

All purple, barren, nubbin, prolific, or multiple-eared plants, or those with blackened bundles fall into the group under discussion. It should be mentioned that these symptoms rarely occur singly, but are grouped

in various ways in single plants. Indications are that certain of these symptoms may result from different causative agents and also that all of them may be caused by one agent.

Lack of pollination results in barren plants no matter what the cause, and usually all barren plants observed in commercial fields turn purple. Corn plants attacked by smut often turn purple and are sometimes barren. The physiologic factors accompanying loss of vigor by selfing sometimes bring out this color symptom. Other factors disturbing the natural physiologic processes of corn plants at critical stages in their growth may cause purple colorations. For example, one of these may be demonstrated by breaking off the ears when in the milk stage. An experiment bearing on this was conducted in 1921, the results of which are given in Table IX.

TABLE IX.—*Number and percentage of Yellow Dent corn plants that turned purple following removal of ears when in milk stage in comparison with uninjured plants in corresponding row, the corn being planted May 27, 1921, near Bloomington, Ill., de-eared August 10, and data recorded September 9*

Rows.	Plants.		
	Total number.	Purpled.	
		Number.	Per cent.
Not de-eared	272	11	4.0
De-eared	272	118	43.4

In the progress of the investigations it has been found that the fungus *Cephalosporium acremonium* Corda (*9, p. 11*) is one of the most important causes in producing the group of symptoms discussed earlier. Occasionally bacteria are found associated with *C. acremonium* in the infected bundles, especially in the lower internodes. In fact, at first it was suspected that bacteria might play an important part in causing certain of these disease manifestations. Even yet, the exact status is not fully clear in this respect, but is being investigated further.

Whether or not the bacteria play a part in the complex, it has been found that *Cephalosporium acremonium* by itself is definitely a parasite capable of producing characteristic symptoms, and that it is seed-borne.

In 1919 special attention was turned to this group of symptoms and *Cephalosporium acremonium* was isolated consistently from blackened bundles of purple-leaf plants. Inoculations with this organism at the bases of dent, flint, and sweet corn when about a foot in height caused blackened bundles in the green leaves and in the stalks. From these leaves and stalks isolations were made and identified as the organism used in the inoculations. Inoculations in the field at Bloomington, Ill., and in the greenhouses at the University of Wisconsin and the University of Illinois in the following winter proved beyond doubt the pathogenicity of this organism; but not until 1921 were any inoculations made with pure cultures in the field from which reliable yield data could be secured. The methods of inoculation and the results are presented in another part of the paper.

In the light of subsequent experience the consistent association of this organism with purple-leaf plants in 1919 was due no doubt to the fact that all the material was collected at one place, Bloomington, Ill., and that practically all of the purple plants produced there in that season were due to the effect of this organism. No such consistency of association was found during 1920 from material collected at the same place, but again in 1921 *Cephalosporium acremonium* was found to be present in a high percentage of isolations from plants of both dent and sweet corn having black vascular bundles. The group of symptoms under discussion may occur to some extent without the presence of *C. acremonium*. Only careful examinations and analyses can determine when this organism is responsible for these symptoms. It is also true that infections by this organism may be quite general in corn without the presence of any of these symptoms to a marked extent, except the blackened fibrovascular bundles.

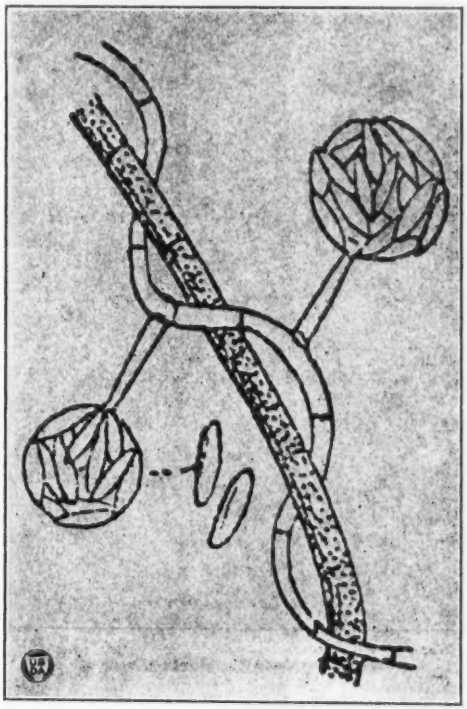

FIG. 2.—After Massee (*33, p. 274, fig. 23*) *Cephalosporium acremonium* twining around a black mold.

THE ORGANISM

The genus Cephalosporium Corda (*9, p. 11*) is characterized by well-developed hyaline mycelium and slender unbranched conidiophores, the spores of which are borne singly at the apex but are pressed to the side by the next produced and eventually form a head held together by mucilage. According to Buchanan (*5*) the genus Hyalopus Corda is differentiated from Cephalosporium solely by the more abundant production of mucilage by the former and the resultant globular refractive head produced. He also concurs with Lindau's (*28, p. 100–101*) characterization of Hyalopus as a Cephalosporium grown in a moist atmosphere, and he also thinks it possible that Allantospora Wakk. is but a growth form of Cephalosporium. Massee (*33, p. 274, 292*), (fig. 2), quoting from Grove (*18*), distinguishes Cephalosporium from Botryosporium by the creeping primary hyphae and by the absence of distinct conidiophores at the tips of the branchlets. It differs from Acremonium by having more than one spore borne at the end of each conidiophore.

Cephalosporium acremonium Corda (*9*), (fig. 3), was described and figured by Corda in 1839, giving it somewhat wide limitations so that it includes any Cephalosporium with small simple oblong or ovoid spores. He found it on dead insects and fungi. The spores figured by him appear too broad to be identical with the species described later and indicate that the organism dealt with in the original description may be identical with *C. acremonium* form *major* described by Penzig (*38*) and later by Grove (*18*). The spore measurements reported for the species and subspecies of *C. acremonium* are presented in Table X.

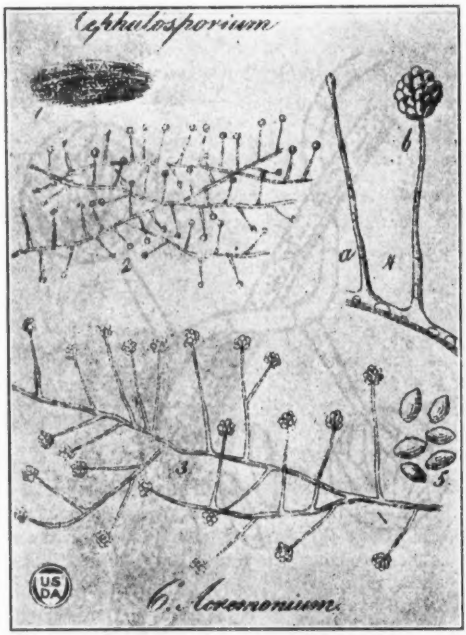

FIG. 3.—After Corda (*9, t. 3, p. 11, pl. 2, fig. 29*

1. Colony.
2. Few plants much enlarged.
3. Single plant greatly enlarged.
4. Piece of hypha with two sporophores, one bearing a spore-head greatly enlarged.
5. Spores greatly enlarged.

Dietrich, 1848 (*12, p. 157*), and Bonorden, 1851 (*3, p. 108*), gave almost the same description and like Corda did not give spore measurements. The first report of the fungus on corn was by Fresenius in 1863 (*17, p. 94–95*), (fig. 4), who reported it on the drying leaves. He gave a careful description, spore measurements, and figures, and retained the name *C. acremonium*. Since his description is entirely in accord with the findings of the authors, a translation of it is presented later in this paper. Rivolta (*41*) in 1873 figured it together with other fungi in his study of plant parasites in their influence on digestion in domestic animals. Saccardo, 1878 (*44, p. 271*), reported it parasitic on *Hypoxylon purpurea*. Penzig, 1882 (*38*), reported a Cephalosporium parasitic on *Alternaria tenuis* in leaves of *Citrus aurantium* and classified it as *C. acremonium* Corda form *major* Penzig. He thought it probably identical with *Acrostalagmus albus* Pr. The latter (*40, p. 126*) was found on dead insects in leaves of *Citrus limonum*. Saccardo, 1881 (*43*) and 1886 (*45*), Costantin, 1888 (*10, p. 91*), Lindau, 1889 (*26, 27, 28*), Sorauer, 1908 (*48*), Ferraris, 1910 (*16, p. 615*), and Oudemans, 1919 (*35, p. 692*), described and figured or gave references to this fungus. Lindau named another fungus host, *Chloridium gigantium*. Massee, 1887 (*32*), found a Cephalosporium parasitic in hyphae of *Heterosporium colocasiae* and named it *C. acremonium* Corda var. *uniseptatum* Massee. Oudemans and Koning, 1902 (*36*), found *C. acremonium* in forest humous soil in Holland. Their careful descrip-

tion is published in English by Jensen, 1912 (*25*). Dop, 1906 (*13*), described a Cephalosporiumlike fungus upon the insect *Aspidiotus perniciosus* on the leaves of *Cocos nucifera* in the island of Martinique. He named it *Hyalopus yvonis*. The description and spore measurements are in accord with *C. acremonium*. Bainier, 1907 (*1*), cultivated, described, and figured the fungus, as did Dale, 1914 (*11, v. 12, p. 56*), in working with soil fungi. She thinks also that the form determined in her first paper (*11, v. 10, p. 465*) as Verticillium is really *C. acremonium*. Peyronel, 1914 (*39*), found *C. acremonium* now and then in the air on high mountains. Fawcett, 1915 (*15*), reported a *Cephalosporium* sp. as the cause of "zoned" leaf spot of coffee. Zimmerman, 1916 (*50*), reported the fungus as a parasite of scale insects on coffee but later gave it the name of *C. lecanii*. Ciferri in Italy, 1921 (*8*), reported *Aspergillus varians* growing on kernels of maize that had developed on the extremities of the ears of plants growing in soil of low humidity. *C. acremonium* was frequently found by him as a parasite on the Aspergillus. Manns and Adams, 1921 (*29, 30, 31*), made investigations upon fungi carried within the kernels of seed corn. They found that 39.54 per cent of Delaware seed corn for planting in 1921 was affected internally with a species of Cephalosporium which they determined as *C. sacchari* Butler and Khan. Butler and Khan (7) described *C. sacchari* as the cause of a wilt of sugar cane in India. The spores of this species are larger than those of *C. acremonium* and often become 1 to 3 septate before germinating. The authors found, upon examination of cultures kindly supplied by Manns and Adams, that the Manns and Adams Cephalosporium does not correspond to the species *sacchari* but to *acremonium* as described by Fresenius (*17, p. 94–95*), and therefore to the organism with which the authors are working. Branstetter, 1922 (*4*), followed Manns and Adams in the identification of the fungus on corn as *Cephalosporium sacchari*. Relative to the fungi carried within the kernels, he says:

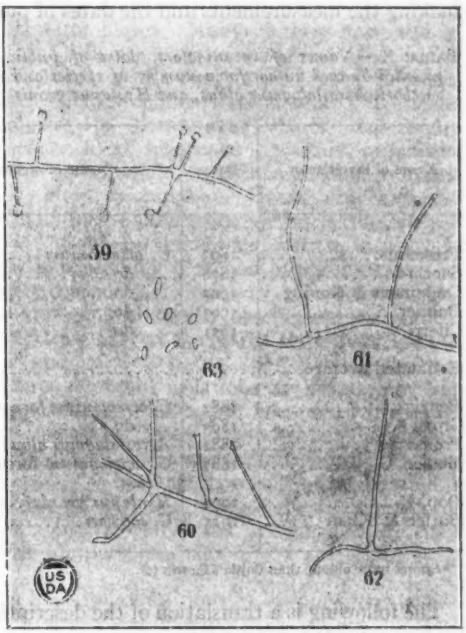

FIG. 4.—After Fresenius (*17, Hft. 3, pl. 11, fig. 59–62*).

59. Piece of mycelium with sporeheads.
60–62. Mycelium with conidophores but no spores.
63. Spores.

> The disease survey shows that 1921 Missouri corn was heavily infected with *Fusarium moniliforme, Cephalosporium sacchari* and *Diplodia zeae* in the order named.

He also states that both *F. moniforme* and *C. sacchari* grew out of many platings and that in these cases only the organism that appeared first was considered. As *F. moniliforme* is a faster-growing organism than the so-called *C. sacchari*, the above statement on the relative occurrence of these two organisms may be misleading.

In comparing closely related species the size of the spores is a very important character. For this reason the spore measurements of *C. acremonium*, *C. sacchari*, *Acrostalagmus albus*, and *Hyalopus yvonis* are presented in Table X, which also includes the names of the investigators making the measurements and the dates of publication.

TABLE X.—*Names of investigators, dates of publications, and spore measurements recorded by each author for a number of species and subspecies of C. acremonium, C. sacchari, Acrostalagmus albus, and Hyalopus yvonis*

Name of investigator.	Date.	Name of organism.	Spore measurements.	
			Length.	Breadth.
			Microns.	*Microns.*
Fresenius	1863	*C. acremonium*	3.3–6	.....
Saccardo	1878	.....do	4	1
Oudemans & Koning	1902	.....do	4	1–1.5
Bainier	1907	.....do	2.5–5	.....
Ciferri	1921	.....do	4.8–6	1.8
Estimated average	.....	.....	4.5	1.35
Penzig	1882	*C. acremonium form major*	4.5–5	2–2.5
Grove [a]	1893	.....do	4–5	2
Preuss	1882	*Acrostalagmus albus*	3.3–3.5	1–1.5
Massee, G.	1887	*C. acremonium* form *unisepta-tum.*	10	4
Dop, P.	1906	*Hyalopus yvonis*	4	1–1.5
Butler & Khan	1913	*C. sacchari*	4–12	2–3

[a] Spores more oblong than Corda's figures (9).

The following is a translation of the description of *C. acremonium* Corda by Fresenius (*17, p. 94–95*):

On leaves of *Zea mais*, which became moist while being dried.

Delicate white mold. From creeping branched mycelium, which by strong magnification does not show septation plainly, arises, like the stalks in a spindle of a raceme, simple, nonseptate, conidiophores of practically equal length, which, at times plainly swollen at the base, gradually taper to a point like an awl and each bears a round head consisting of many spores held together by slime. These conidiophores develop either singly or crowded almost in whorls; they occur also with two terminal spore heads as in Figures 59 and 60; in the latter two very short projections can be seen which indicate that, in this case, both heads stood very close together. When placed in a drop of water, the spore heads dissolve immediately and the spores are dispersed. The spores vary considerably in size and form; they measure 1/300–1/170 mm. in length and, in outline, vary in shape from oval to oblong or almost linear (Fig. 4).

The general habit of the fungus strongly suggests that of Acremonium; except in the latter genus only a single spore is borne on the end of each conidiophore. The conidiophores with their spore heads are somewhat similar to Hyalopus.

Norton and Chen (*34*) reported a corn seed parasite in 1920 which they thought may have been described as Oospora, Cephalosporium, Acrostalagmus, or Verticillium. The occurrence of spores that are sometimes

septate and conform in size to the microconidia of Sheldon's *Fusarium moniliforme* (47) would differentiate it from *C. acremonium*.

ISOLATION OF ORGANISM AND GROWTH ON ARTIFICIAL MEDIA

The usual method of isolating the organism from the stalks was as follows: The stalk, while still turgid, was cut obliquely near the base to determine the presence of blackened bundles. If these bundles were present, pieces of the internodes were selected, split lengthwise, and immediately small portions of the blackened bundles were transferred aseptically to potato-agar slants or plates. Many of these gave rise to apparently pure cultures of *Cephalosporium acremonium* and, upon abundant sporulation, small pieces of the fungus were used to make spore suspensions from which dilution plates were poured. Transfers were made from single colonies to potato-agar slants. Internodes of the stalks are used because local infections by other organisms are somewhat common at the nodes, while organisms causing systemic infections are comparatively few in number. Hence, the different organisms to be found internally in the internodes are not abundant.

Cephalosporium acremonium grows on numerous culture media. It makes rapid progress on media containing a carbon compound, such as dextrose, maltose, lactose, saccharose, mannit, or glycerine. Aerial mycelia with conidiophores and conidia usually are present on potato-dextrose agar and nutrient-dextrose agar at the end of three days' growth at temperatures between 17° and 32° C.

The hyphae in young cultures of recent isolations are in more or less loose cottony masses but appear somewhat coarse because of their tendency to agglutinate. The mycelium forms a moderately thick pale pink skin on the substrate by anastomosis and agglutination. It does not change the color of the agar. When the organism is first isolated it is usually pink or salmon colored and spore production is abundant. When carried for long periods on artificial media, it soon loses its color and more slowly it decreases in spore production and the quantity of aerial mycelia.

Some 3-year old isolations have no aerial mycelia except a few spikes of agglutinated hyphae which often form in rosettelike groups. Macroscopically, various isolations show some differences in culture, particularly in the intensity of the pink coloration and in the height and uniformity of the hyphae bearing the conidiophores. Isolations from sweet corn as a rule tend to be pinker in color and the little fascicles of aerial hyphae are more irregular and more curled than they are in isolations from dent corn. The spores are borne singly at the end of the conidiophore and collect in a mucilaginous ball containing from a few to as many as 50 or more. The fluid in which they are involved is copious enough to obscure any outline of the spores at the periphery of the sphere, giving them the appearance of glistening drops. When the spore head is placed in water the spores disperse immediately, but each spore retains a capsule. On account of this capsule, measurements of the spores vary with the moisture of the medium on which they are grown and with the manner in which they are mounted for examination. In India ink, allowed to dry at room temperature and examined immediately, they measure 3–6 by 1–1.8 microns and average 4.3 by 1.3 microns (Pl. 6, B, C, D). These measurements apply to isolations from both sweet and dent corn and also to the organism supplied by Manns and Adams.

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Indicator media containing dextrose, saccharose, and glycerine, respectively, with cresol purple to detect increase in acidity and cresol red to detect increase in alkalinity were used in an attempt further to classify the various isolations of *Cephalosporium acremonium*. No data were obtained that would separate the sweet corn from the dent corn isolations. Although each isolation behaved consistently in a number of trials, it was found that two isolations from the same stalk might differ in their behavior on these media. Work is being continued on the organism, but for the present the various isolations, differing in minor characteristics, are regarded as various strains of *C. acremonium*. The organism supplied by Manns and Adams is also considered as representing this species rather than *C. sacchari*.

LIFE CYCLE OF CEPHALOSPORIUM ACREMONIUM

Infected ears carry the organism internally in the seed. As will be shown later, *Cephalosporium acremonium* is definitely pathogenic. It develops with the germinating kernel and causes a systemic infection of the plant through the vascular system (Pl. 6, A). By this means, it invades the ears and eventually the kernels. In this manner it is carried over to the following season. Occasionally an ear can be found which is externally overrun. In observations extending over a period of three years, only one such ear has been found. Probably an especially damp harvest season or poor storage conditions are necessary for this development. It seems possible that with such development, the infection might spread from ear to ear. Extensive inoculation experiments in which spore suspensions were placed on the seed gave, on the whole, negative results (see Table XI). However, the question of soil infection has not been determined definitely as yet.

Seed infection can be determined microscopically on limestone-sawdust germinators as described by Holbert and Hoffer (*21, p. 14–16*), but the symptoms are easily overlooked so that up to the present only a fair degree of accuracy has been attained by this method.

Infected germinating kernels have blanched, white tips, sometimes with noticeable mycelial growth. Often, however, the mycelial growth can not be seen until examined microscopically. When the symptoms on the germinator are overlooked the ears often are chosen for seed, because germination and the vigor of the infected seedlings are seldom impaired at this time.

In experiments, macroscopic examination of germinating kernels is followed by microscopic examination of those suspected. In this way the infected ears can be accurately determined (Pl. 6, E). In two instances 30 ears of this type were obtained. Two composites made from an equal number of kernels from each of these ears showed 65 per cent and 95 per cent Cephalosporium infections, respectively.

Cephalosporium acremonium has been isolated from black bundles in first leaves (Pl. 6, F), from bundles in the stalks (Pl. 1 (right), 3 B), from the shanks, from the cobs, and from the kernels.

INOCULATION EXPERIMENTS WITH CEPHALOSPORIUM ACREMONIUM

These experiments were conducted near Bloomington, Ill., on uniform, well-drained, fertile brown silt loam soil. The land on which the inoculation experiments were located never had been cropped previously.

TABLE XI.—*Field and harvest data on the influence of seed inoculation with Cephalosporium acremonium. Seed inoculations were made at time of planting, May 11, 1921. Various strains of Yellow Dent corn grown on virgin, brown silt loam soil, Bloomington, Ill.*

Plat No.	Field stand.				Suckers.		Purple stalks.		Prolific stalks.		Preharvest count of ears produced.				Barren stalks.		Sound marketable ears (by count after harvest).		Acre yield of shelled corn (corrected to uniform moisture basis).				Reduction in acre yields of inoculated plats.			
											Good ears.		Nubbin ears.						Total yield.		Yield of sound marketable corn.		Total yield.		Yield sound marketable corn.	
	Control.		Inoculated.		Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.								
	No.	*P. ct.*	*No.*	*P. ct.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*P. ct.*	*Bu.*	*P. ct.*
1	58	96.7	58	96.7	2	10	2	1	1	2	55	51	4	0	4	6	38	49	107.8	105.6	83.9	89.3	2.2	2.0	+5.4	+6.4
2	57	95.0	57	95.0	10	9	3	0	3	6	45	39	5	5	11	15	30	28	95.4	87.9	68.7	57.6	7.5	7.9	11.1	16.2
3	58	96.7	59	98.3	10	10	5	2	2	8	55	48	2	3	6	9	41	39	104.0	96.7	82.7	74.6	7.3	7.0	8.1	9.8
4	53	88.3	53	88.3	19	20	1	0	4	4	43	41	6	4	10	15	30	25	88.9	91.2	66.7	53.9	+2.3	+2.6	12.8	19.2
5	57	95.0	58	96.7	13	13	4	0	1	2	48	47	4	2	9	10	39	37	98.6	99.7	76.3	71.6	+1.1	+1.1	4.7	6.2
6	56	93.3	53	88.3	10	10	2	2	1	4	52	46	2	4	6	7	35	34	98.3	96.5	69.6	66.5	1.8	1.8	3.1	4.5
7	59	98.3	58	96.7	6	9	1	0	2	3	48	48	4	6	9	6	33	35	95.5	99.7	71.6	68.3	+4.2	+4.4	3.3	4.6
8	56	93.3	56	93.3	13	9	1	0	0	5	50	43	2	5	4	11	33	36	93.2	99.7	63.9	74.2	+6.5	+7.0	+10.3	+16.1
9	57	95.0	53	96.7	16	11	2	1	4	2	45	50	4	5	10	4	34	37	87.0	96.7	67.6	70.5	+9.7	+11.1	+2.9	+4.3
10	57	95.0	59	98.3	6	18	1	1	1	5	44	42	9	15	8	7	31	27	87.5	90.8	57.9	49.5	+3.3	+3.8	8.4	14.5
11	58	96.7	58	96.7	15	15	11	5	3	3	51	44	3	6	7	10	37	37	97.2	77.9	72.2	74.9	19.3	19.9	+2.7	+3.7
12	57	95.0	56	93.3	22	15	1	3	1	4	42	39	7	8	10	12	28	29	81.4	82.0	51.9	51.7	+0.6	+0.7	0.2	0.4
13	58	96.7	60	100.0	12	12	3	1	4	1	45	49	4	6	11	6	34	36	86.7	94.2	62.7	66.8	+7.4	+8.5	+4.1	+6.5
14	56	93.3	56	93.3	7	14	2	0	1	2	47	46	4	6	6	7	32	35	73.5	86.0	53.9	60.2	+12.5	+17.0	+6.3	+11.7
15	58	96.7	55	91.7	15	11	6	7	4	4	42	45	4	2	14	12	33	32	94.7	84.9	72.3	59.8	9.8	10.3	12.5	17.3
16	57	95.0	56	93.3	8	4	5	1	2	1	40	41	4	8	11	8	26	26	70.9	73.8	44.1	42.8	+2.9	+4.1	1.3	2.9
17	54	90.0	51	85.0	5	7	6	2	4	3	40	42	7	4	9	11	31	40	74.6	84.2	55.9	72.4	+9.6	+12.9	+16.5	+29.5
18	56	93.3	48	80.0	4	5	6	3	4	1	37	38	9	7	11	11	28	22	72.4	72.7	48.9	42.8	+0.3	+0.4	6.1	12.5
19	58	96.7	56	93.3	6	5	9	5	1	7	46	41	7	6	9	14	32	26	78.7	74.6	56.3	45.4	4.1	5.2	10.9	19.4
20	54	90.0	54	90.0	5	6	4	2	2	3	42	39	5	9	14	7	23	27	72.0	79.4	41.6	49.8	+7.4	+10.3	+8.2	+19.7
Total	1,134	.....	1,119	.....	204	213	75	36	45	70	917	879	96	106	179	188	648	657	1,758.3	1,774.1	1,268.7	1,242.6	.....	.....	.....	.....
Per cent increase following inoculation	.....	.....	.....	.....	.....	4.4	.....	.....	.....	55.6	.....	.....	.....	10.4	.....	5.03	.....	1.4	.....	0.9	.....	.....	.....	.....	.....	.....
Per cent decrease following inoculation	.....	.....	1.3	.....	.....	.....	.....	52	.....	.....	.....	4.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.06	.....	.....	.....	.....

TABLE XII.—*Data showing comparative field stand, number of suckers, purple stalks, barren stalks, and total and marketable ears, and yields of shelled corn from various strains of Yellow Dent corn, inoculated hypodermically when about 12 inches high with Cephalosporium acremonium and from controls injected with corresponding amounts of sterile water, planted May 11, 1921, in adjacent plats on uniform virgin brown silt loam soil, near Bloomington, Ill.*

Plat No.	Final field stand.				Suckers.		Purple stalks.		Prolific stalks.		Preharvest count of ears produced.				Barren stalks.		Sound, marketable ears (by count after harvest.)		Acre yield of shelled corn (corrected to uniform moisture basis).			
											Good ears.		Nubbin ears.						Total yield.		Yield of marketable corn.	
	Control.		Inoculated.																			
	Number.	Per cent.	Number.	Per cent.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.
																			Bushels.	Bushels.	Bushels.	Bushels.
1	56	93.3	57	95.0	19	32	0	0	1	4	53	44	6	6	4	10	40	33	106.0	91.6	77.5	62.4
2	58	96.7	56	93.3	6	37	0	1	3	3	45	37	7	7	11	15	30	22	98.9	77.2	63.9	47.3
3	59	98.3	57	95.0	14	51	0	2	0	2	56	39	1	8	5	14	44	30	102.3	85.7	81.2	59.8
4	56	93.3	50	83.3	18	44	1	0	3	7	43	35	7	12	9	15	31	19	89.5	75.7	62.9	41.0
5	59	98.3	56	93.3	13	21	0	0	0	6	51	45	2	3	7	9	42	33	104.1	86.0	84.9	64.6
6	57	95.0	59	98.3	16	29	3	5	1	2	54	45	2	6	3	8	31	31	97.8	92.3	56.5	63.1
7	58	96.7	59	98.3	22	35	2	0	1	4	49	47	7	9	6	9	39	33	99.7	89.0	78.3	61.3
8	57	95.0	59	98.3	11	26	0	1	0	3	54	41	1	6	3	16	37	28	95.2	86.0	66.5	54.6
9	59	98.3	59	98.3	20	37	0	0	3	7	46	37	5	7	9	13	35	30	90.1	83.8	65.3	56.5
10	58	96.7	59	98.3	7	38	0	2	0	5	46	33	11	15	7	16	31	21	93.8	70.9	58.7	38.4
11	59	98.3	52	86.7	15	48	1	1	4	4	50	38	2	8	9	12	38	33	95.6	84.5	73.5	64.2
12	56	93.3	56	93.3	21	50	2	1	1	3	38	29	6	8	16	20	25	19	73.1	57.5	44.3	31.4
13	59	98.3	54	90.0	16	38	2	3	5	5	48	36	2	2	10	18	36	27	92.3	73.8	69.4	52.0
14	53	88.3	60	100.0	10	39	1	2	1	1	41	43	7	7	6	12	31	28	78.3	78.3	51.3	47.6
15	59	98.3	60	100.0	8	36	5	12	4	7	45	42	1	6	12	12	36	32	110.8	79.7	90.4	57.2
16	59	98.3	58	96.7	20	18	3	3	3	2	43	46	7	8	9	10	27	25	72.0	78.3	43.2	41.0
17	59	98.3	59	98.3	10	28	2	3	5	8	43	36	7	8	9	16	30	20	76.8	65.3	52.1	35.1
18	57	95.0	55	91.7	13	25	5	5	3	8	32	25	13	15	14	19	25	16	65.3	57.2	41.7	30.3
19	59	98.3	57	95.0	20	33	2	3	1	6	45	30	6	13	12	16	34	19	80.1	60.9	59.8	35.1
20	54	90.0	53	88.3	24	37	5	10	1	7	42	27	7	11	9	19	26	17	72.0	55.4	47.6	28.8
21	58	96.7	55	91.7	8	20	1	2	2	5	40	30	7	11	12	14	28	22	70.0	57.9	47.6	38.4
22	51	85.0	54	90.0	11	42	3	5	1	5	35	39	10	6	11	15	26	30	65.0	73.8	47.3	53.9
23	54	90.0	57	95.0	16	48	2	7	2	1	42	40	7	17	7	13	34	31	62.4	78.3	60.2	54.6
24	48	80.0	53	88.3	3	29	2	8	6	7	34	34	8	6	7	16	26	18	70.5	62.0	51.7	35.8
25	51	85.0	60	100.0	21	52	0	5	3	3	42	41	7	6	9	14	30	33	84.9	83.1	54.6	56.1
26	51	85.0	46	76.7	10	26	3	2	2	6	34	34	6	10	12	9	19	14	70.5	65.3	39.1	28.1
27	56	93.3	50	83.3	13	53	7	1	1	2	37	38	10	8	11	12	28	25	76.8	70.9	55.4	43.9
28	52	86.7	55	91.7	21	22	3	2	1	2	45	39	4	6	10	13	21	25	79.4	83.4	42.5	53.5
Mean	56.1	93.6	55.9	93.1	14.5	35.5	2.0	3.1	2.1	4.5	44.0	37.5	5.9	8.4	8.9	13.8	31.4	25.1	84.8	75.1	59.6	47.7

The plats were hand-planted in hills 42 inches apart each way at the rate of 3 kernels per hill. Great care was exercised throughout the season to avoid mechanical injury of the plants during cultivation, and to guard against insect pests and rodents, but no attempt was made either to thin to a uniform stand or to correct for differences in stand.

Acre yields have been reduced to a uniform moisture basis of 14 per cent and reported as "Total yield" and "Yield of marketable corn," the latter obtained from the former by excluding small nubbins, rotten ears, and light chaffy ears as described by Holbert et al (*22*).

Table XI gives the field and harvest data on the influence of inoculating various strains of Yellow Dent corn with *Cephalosporium acremonium* isolated from dent corn. The inoculum was in the form of a spore suspension in sterile water, enough spores from potato-dextrose agar cultures being used to produce a moderate cloudiness in the water. The seed was soaked in the inoculum not to exceed one-half hour and then planted.

As was noted above under life cycle of the organism, Table XI is a presentation of data on seed inoculation. The effects in this case are almost negative.

Table XII gives the results of inoculations with *Cephalosporium acremonium* with all conditions similar to those in Table XI except that the inoculations were made by means of a hypodermic syringe when the plants were about 12 inches in height. Approximately 0.2 cc. of the suspension of the organism was injected into the central portion of each stalk at about 2 inches above the crown of the plant. Control plants were injected with corresponding amounts of sterile water. The difference in final field stands of the inoculated plats and the control plats averaged less than one-half per cent.

TABLE XIII.—*Summary of data presented in Table XII*

Points of comparison.	Control.	Inoculated.	Per cent increase following inoculation.	Per cent decrease following inoculation.	Odds.
Mean percentage final field stand	93.6	93.1	.....	0.5	.....
Mean number of suckers per plat	14.5	35.5	144.8	.....	> 999:1
Mean number of purple stalks per plat	2.0	3.1	55.0	.....	74:1
Mean number of prolific stalks per plat	2.1	4.5	114.3	.....	> 999:1
Mean number of barren stalks per plat	8.9	13.8	55.1	.....	> 999:1
Mean number of nubbin ears per plat	5.9	8.4	42.4	.....	> 999:1
Mean total yield (bushel) per plat	84.8	75.1	.....	11.4	> 999:1
Mean yield of marketable corn (bushel) per plat	59.6	47.7	.....	20.0	> 999:1

It will be noted from Table XIII, which gives a summary of the data in Table XII, that there was a 144.8 per cent increase in number of suckers, 55 per cent increase in purple stalks, 114.3 per cent increase in prolific stalks, 55.1 per cent increase in number of barren stalks, 42.4 per cent increase in number of nubbins, 11.4 per cent decrease in total yield, and 20.0 per cent decrease in yield of marketable corn in the plats inoculated with *Cephalosporium acremonium*. The increase in the number of suckers on the inoculated plants was the most apparent symptom in the plats. This

also was the case in 1922 both in inoculated plants from horny, nearly disease-free seed (Pl. 5 A, B, C) and in ear rows from naturally infected seed (Pl. 4 A, B). However, inoculations of various strains of corn with this organism do not produce this symptom consistently. Starchy composites, especially, show less tendency to sucker when inoculated with this organism, but the main stalks are more markedly affected, resulting in numerous weak, spindly, low-yielding plants (Pl. 5 C, D). In this latter case the reduction in yield generally is greater than in better strains of corn which show increase of suckering.

Table XIV gives the results of inoculations with *Cephalosporium acremonium* with all conditions similar to those described in Table XII, except that the date of planting, May 28, was later, that the control plants were not injected with sterile water, and that five rows in a third series were inoculated with *Aplanobacter stewarti* (E F S) McCul. This bacterial organism, also pathogenic on dent corn, is further similar to *C. acremonium* in its attack on corn in that it causes vascular infections of the plants. Yields of the plats, the plants of which were injected with sterile water, were compared with the yield from a number of corresponding control plats not thus injected. The injections of water seemed to have no effect. In every case the yields from the group of plants receiving sterile water injections equaled or slightly exceeded the corresponding ones not thus injected. Therefore, mechanical injury caused by the inoculations was considered negligible.

In agreement with the results given in Tables XII and XIII, the results given in Tables XIV and XV show an increase of suckers, purple stalks, prolific stalks, nubbin ears, and barren stalks, and a significant decrease in good ears and yield from the plants inoculated with *Cephalosporium acremonium*.

Comparing the inoculations of the two parasites, *Cephalosporium acremonium* increased the number of purple stalks (152.4 per cent) while *Aplanobacter stewarti* did not increase them, and although *Aplanobacter stewarti* increased suckering, the *C. acremonium* plats averaged 110.8 per cent more suckers than the *A. stewarti* plat. In a similar way there were 55.3 per cent more nubbin ears in the *C. acremonium* plats than in the *A. stewarti* plats. The number of barren stalks was increased and the yields decreased about the same amount in both inoculations. Therefore, the outstanding differences in the effects of those two pathogenes causing vascular diseases of corn are the increase in the number of purple stalks and the larger number of suckers, prolific stalks, and nubbin ears caused by *C. acremonium*.

In Tables XII to XV it will be noted that the reductions in yield due to the hypodermic inoculations with *Cephalosporium acremonium* and *Aplanobacter stewarti* range from slight increases to as much as 44.6 per cent reduction in acre yields of marketable corn. Table XVI presents data comparing the resistance and susceptibility of different seed selections from various strains of Yellow Dent corn to the vascular diseases induced by *C. acremonium* and *A. stewarti*. Data from inoculations with *Gibberella saubinetii* on corn grown from similar seed from the same sources are included to show that, in general, seed lots susceptible to these two vascular diseases are likewise susceptible to one of the rootrot organisms.

TABLE XIV.—*Data showing comparative field stand, number of suckers, purple stalks, barren stalks, and total and marketable ears, and yields of shelled corn from various strains of Yellow Dent corn uninoculated and inoculated hypodermically when about 12 inches high with Aplanobacter stewarti and Cephalosporium acremonium, respectively, planted May 28, 1921, in adjacent plats on uniform, virgin, brown silt loam soil, at Bloomington, Ill.*

Plat No.	Final field stand (60 plants perfect stand).						Suckers.			Purple stalks.			Profilic stalks.			Preharvest count of ears produced.						Barren stalks.			Sound marketable ears (by count after harvest).			Acre yield of shelled corn (corrected to uniform moisture basis).					
																Good ears.			Nubbin ears.									Total yield.			Yield of marketable corn.		
	Control.		*A. stewarti.*		*C. acremonium.*		Control.	*A. stewarti.*	*C. acremonium.*	Control.	*A. stewarti.*	*C. acremonium.*	Control.	*A. stewarti.*	*C. acremonium.*	Control.	*A. stewarti.*	*C. acremonium.*	Control.	*A. stewarti.*	*C. acremonium.*	Control.	*A. stewarti.*	*C. acremonium.*	Control.	*A. stewarti.*	*A. acremonium.*	Control.	*A. stewarti.*	*C. acremonium.*	Control.	*A. stewarti.*	*C. acremonium.*
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*
1	60	100.0	59	98.3	59	98.3	6	16	31	1	2	4	0	1	5	53	44	46	1	7	8	5	8	10	38	33	36	91.9	84.9	90.1	70.5	63.7	64.2
2	60	100.0	60	100.0	56	93.3	7	13	26	2	3	5	2	5	4	46	44	38	1	2	8	13	14	12	25	18	27	84.2	73.3	79.0	51.5	32.3	53.5
3	60	100.0	59	98.3	60	100.0	5	13	28	1	2	10	0	1	4	42	48	44	2	5	6	7	6	10	40	39	38	94.1	84.0	91.5	75.1	72.4	71.9
4	58	96.7	58	96.7	57	95.0	9	14	37	4	4	10	1	3	2	45	42	44	3	5	7	9	14	8	28	12	29	90.6	65.3	84.9	54.6	25.8	56.5
5	60	100.0	60	100.0	60	100.0	1	8	33	0	2	9	4	2	3	51	45	45	1	6	5	8	8	12	39	29	38	84.7	87.9	89.3	72.4	58.7	71.6
6	57	95.0	56	93.3	57	95.0	1	22	40	4	2	7	0	2	3	46	39	40	2	6	5	10	16	17	28	23	25	74.4	69.8	72.7	51.5	49.7	47.6
7	60	100.0	59	98.3	60	100.0	2	10	34	5	3	6	0	1	0	50	47	49	1	2	9	5	10	5	40	37	41	86.4	81.8	101.1	73.7	68.7	71.6
8	59	98.3	59	98.3	54	90.0	5	12	33	5	7	10	3	3	5	47	38	42	3	6	3	9	17	16	33	21	27	83.6	75.1	79.7	59.1	41.4	53.5
9	59	98.3	59	98.3	59	98.3	7	12	37	5	3	4	2	3	2	48	50	47	3	1	7	8	8	7	37	35	35	91.7	88.2	93.0	75.9	63.7	69.4
10	59	98.3	59	98.3	59	98.3	6	9	26	4	1	4	5	2	2	45	43	45	2	6	6	13	11	12	24	31	24	72.5	80.3	80.5	43.2	52.4	42.4
11	59	98.3	59	98.3	58	96.7	11	29	32	1	6	0	0	4	3	52	54	51	1	1	5	8	8	7	38	34	37	93.6	93.4	93.8	72.7	63.5	66.1
12	58	96.7	58	96.7	57	95.0	7	15	35	1	0	5	3	2	0	48	47	42	4	6	7	6	8	10	34	29	32	87.7	82.5	80.8	61.1	53.5	53.9
13	59	98.3	60	100.0	58	96.7	0	14	48	2	0	10	1	2	4	54	47	45	3	2	7	3	11	11	47	37	38	96.9	86.8	91.1	87.1	67.4	70.8
14	59	98.3	58	96.7	57	95.0	1	10	28	0	0	3	1	3	0	49	43	47	1	6	4	9	11	8	36	36	34	84.4	93.6	83.1	64.6	59.8	60.9
15	57	95.0	59	98.3	59	98.3	0	11	27	1	0	7	1	3	4	50	47	43	1	6	6	5	9	13	40	32	38	82.0	84.4	87.1	67.9	63.7	71.2
16	60	100.0	60	100.0	59	98.3	3	12	35	2	1	9	0	0	4	47	51	45	5	3	8	7	6	12	37	32	33	82.1	86.2	79.4	60.7	57.6	55.6
17	59	98.3	59	98.3	58	96.7	6	17	36	3	0	5	3	3	0	50	42	51	2	6	5	6	13	5	40	38	43	93.4	93.8	96.7	77.9	70.0	80.8
18	58	96.7	57	95.0	56	93.3	5	30	37	0	1	6	0	3	7	54	42	41	2	5	6	3	14	13	40	26	30	94.0	78.1	77.9	74.8	46.1	54.6
19	59	98.3	59	98.3	59	98.3	14	28	27	2	3	2	4	1	0	45	52	53	2	4	4	13	6	7	45	43	41	98.4	103.9	94.1	77.3	84.2	76.1
20	55	91.7	54	90.0	53	88.3	10	18	46	1	1	0	2	2	5	44	42	42	3	2	6	14	12	12	33	23	27	79.9	65.2	80.5	60.9	43.0	48.7
21	60	100.0	60	100.0	56	93.3	10	15	51	3	0	6	4	0	5	51	57	40	5	0	10	5	3	8	40	38	34	96.4	94.7	82.7	77.0	69.6	61.3
22	60	100.0	60	100.0	56	93.3	2	20	39	0	1	5	0	4	11	53	52	38	4	1	7	3	8	13	38	37	27	93.4	91.7	66.8	68.5	68.5	46.5
23	59	98.3	59	98.3	59	98.3	5	27	37	0	3	2	2	2	1	52	52	47	2	2	10	6	8	4	39	42	38	94.9	96.9	91.2	77.3	79.6	73.8
24	60	100.0	60	100.0	56	93.3	5	10	40	2	1	6	0	3	6	55	50	45	2	3	5	3	8	10	47	39	33	92.5	91.9	81.2	84.7	71.8	60.5
25	60	100.0	59	98.3	60	100.0	4	19	31	1	2	0	0	0	4	51	52	51	4	4	1	4	6	8	45	43	38	99.7	95.4	90.4	84.5	78.1	69.8
26	52	86.7	51	85.0	52	86.7	4	21	16	5	4	8	2	2	5	42	40	38	4	5	8	11	13	13	22	21	15	80.5	75.1	77.5	44.1	45.0	31.0
27	59	98.3	58	96.7	59	98.3	4	16	31	3	0	1	1	1	5	56	50	50	1	3	4	3	5	9	45	36	38	108.7	98.9	89.7	86.2	70.0	70.8
28	59	98.3	58	96.7	58	96.7	4	9	26	2	6	5	2	2	1	42	47	52	6	3	2	11	9	6	31	20	23	97.3	83.3	91.9	58.7	39.5	47.6
29	59	98.3	60	100.0	59	98.3	0	7	20	2	2	4	2	2	1	47	50	46	3	1	3	7	9	10	32	30	33	100.8	72.7	80.8	76.8	53.3	58.0
Mean	58.7	97.9	58.5	97.5	57.6	96.0	5.0	15.8	33.3	2.1	2.1	5.3	1.6	2.1	3.3	48.8	46.8	45.1	2.6	3.8	5.9	7.4	9.6	9.9	36.6	31.5	32.8	90.0	84.8	85.5	68.6	59.1	60.7

TABLE XV.—*Summary of data presented in Table XIV*

Points of Comparison.	Control.	Inoculated.		Increase or decrease following inoculation.			
		A. stewarti.	*C. acremonium.*	*A. stewarti.*		*C. acremonium.*	
				Per cent.	*Odds.*	*Per cent.*	*Odds.*
Mean percentage final field stand	97.9	97.5	96.0	0.4	.....	1.9	.....
Mean number of suckers per plat	5.0	15.8	33.3	216.0	>999:1	566.0	>999:1
Mean number of purple stalks per plat	2.1	2.1	5.3	.....	.....	152.4	>999:1
Mean number of prolific stalks per plat	1.6	2.1	3.3	31.3	15:1	106.3	195:1
Mean number of barren stalks per plat	7.4	9.6	9.9	29.7	554:1	33.8	356:1
Mean number of nubbin ears per plat	2.6	3.8	5.9	46.2	78:1	126.9	>999:1
Mean total acre yield (bushel) per plat	90.0	84.8	85.5	−5.8	554:1	−5.0	199:1
Mean acre yield of marketable corn (bushel) per plat	68.6	59.1	60.7	−13.8	>999:1	−11.5	>999:1

The nearly disease-free control (Table XVI) was a composite of seed ears selected from apparently healthy plants and showing no evidence of rotting or infection when tested on a limestone-sawdust table germinator. This strain of Yellow Dent corn is the result of several years careful plant and germinator selection to avoid seed from plants affected with any of the root, stalk, and ear-rot diseases. Corn grown from this seed was highly resistant to injury following inoculation with *Gibberella saubinetii*, but was damaged to the extent of 12.9 per cent by inoculation with *Cephalosporium acremonium*. Although care had been exercised to avoid plants showing purple leaves and multiple ears, it is probable that many ears were included from year to year that had grown on plants more or less infected with *C. acremonium*. This fact may account for the greater susceptibility of this corn to *C. acremonium* than to *G. saubinetii*. It is also interesting to note that the nearly disease-free control corn was not equally susceptible to the two vascular diseases induced by *C. acremonium* and *A. stewarti*, respectively, being rather susceptible to the former, but highly resistant to the latter.

The other lots of seed referred to in Table XVI were furnished by three representative farmers from three different counties in central Illinois. From these lots of seed, two selections of approximately 300 ears each were chosen on the basis of physical appearance. Ears included in the "good seed" selection had been grown on plants apparently not much affected by any of the root, stalk, and ear-rot diseases, while the ears included in the "susceptible seed" selection bore evidence of having been produced on diseased plants. No dead or weak-germinating ears were included. Data presented in Table XVI show that the corn grown from the susceptible seed selection was affected to a much greater extent, as expressed in terms of acre yield, by the inoculations with both *C. acremonium* and *A. stewarti* than was the corn grown from the good seed selection. Corn grown from the susceptible seed selection also was more susceptible to injury following inoculation with *G. saubinetii*.

TABLE XVI.—*Yield data from certain selections and strains of Yellow Dent corn when inoculated with Cephalosporium acremonium, Aplanobacter stewarti, and Gibberella saubinetii, the corn being grown near Bloomington, Ill., in 1921, on virgin brown silt loam soil of high fertility*

Character of seed.	Inoculated with—	Number of plats.	Total acre yield.		Reduction following inoculation.		Odds.
			Control.	Inoculated.			
			Bushels.	*Bushels.*	*Bushels.*	*Per ct.*	
Susceptible selection	*C. acremonium*	6	84.0	65.7	18.3	21.8	>999:1
Good seed selection	do	6	88.0	81.5	6.5	7.4	45:1
Susceptible selection	*A. stewarti*	6	85.6	74.1	11.5	13.4	59:1
Good seed selection	do	6	84.1	85.0	+0.9	+1.1	1:1
Susceptible selection	*G. saubinetii*	6	84.6	69.0	15.6	18.4	158:1
Good seed selection	do	6	85.7	80.6	5.1	6.0	8:1
Nearly disease-free control	*C. acremonium*	14	89.4	77.9	11.5	12.9	>999:1
Nearly disease-free control	*A. stewarti*	14	93.9	91.1	2.8	3.0	37:1
Nearly disease-free control	*G. saubinetii*	14	90.4	87.5	2.9	3.2	50:1

A further study of the field performance of these same seed lots is given in Table XVII. Corn grown from the susceptible seed selection, which was injured to the extent of 21.8 per cent, 13.4 per cent, and 18.4 per cent by inoculations with pure cultures of *C. acremonium*, *A. stewarti*, and *G. saubinetii*, respectively, yielded 8.6 ± 1.1 bushels less sound corn than corn grown from the good seed selection which was highly resistant to injury from inoculations with all three organisms.

TABLE XVII.—*Yield data from the susceptible and good selections and the nearly disease-free control, grown near Bloomington, Ill., in 1921, on infested brown silt loam soil of medium fertility*

Character of seed.	Number of plats.	Acre yield.		Increase in yield of marketable corn of good seed selection over susceptible selection.			Increase in yield of marketable corn of nearly disease-free check over good seed selection.		
		Total.	Marketable.						
		Bushels.	*Bushels.*	*Bushels.*	*Per cent.*	*Diff. / P. E.*	*Bushels.*	*Per cent.*	*Diff. / P. E.*
Susceptible seed selection	42	74.8±0.5	46.5±0.7	.....	.....	.....	.....	.....	.....
Good seed selection	42	79.6±0.7	55.1±0.8	8.6±1.1	18.5	7.8	.....	.....	.....
Nearly disease-free check	42	86.4±0.5	64.3±0.5	.....	.....	.....	9.2±0.9	16.7	10.2

Additional data on the field performance of the susceptible and good seed selections at various points in Illinois are presented in Table XVIII. Although the difference in yield between these two selections was not large at Bloomington, nor at Peoria, where corn followed clover, yet the mean difference in yield at all points was 11.9 bushels, or 15.4 per cent, with odds of 58:1. The mean difference in yield of marketable corn was larger.

Since these data (Tables XVI, XVII, XVIII) show that resistance to the two vascular diseases (Table XVI) probably is closely associated with the ability to yield well in soil infested with disease-producing

organisms and under unfavorable environment, it is thought that plant breeders may take advantage of this relationship in developing productive strains and varieties of corn more resistant to root, stalk, and ear-rot diseases. Eliminations of homozygous strains on the basis of susceptibility could be made by inoculating with one or both of the vascular disease organisms. The authors also feel that inoculations with *Cephalosporium acremonium* and *Aplanobacter stewarti* would add considerably to the value of corn varietal tests.

TABLE XVIII.—*Yield data of corn grown from good seed and susceptible seed selections, the experiments being conducted on brown silt loam soil at various points in Illinois, in 1921*

Location (Illinois).	Previous crop.	Total acre yield.		Decrease in yield from susceptible seed selection.
		Good seed selection.	Susceptible seed selection.	
		Bu.	*Bu.*	*Bu.*
Bloomington	Virgin prairie sod	88.9	84.5	4.4
Do	Corn	78.4	74.6	3.8
Do	do	83.3	75.8	7.5
Peoria	Clover	73.4	71.5	1.9
Do	Corn	81.5	66.6	14.9
Do	do	83.2	66.7	16.5
Do	do	85.4	62.8	22.6
Virginia	Clover	69.8	50.1	19.7
Do	do	67.0	59.5	7.5
Do	do	64.4	43.8	20.6
Mean		77.5	65.6	[a] 11.9

[a] Odds 58:1.

SUMMARY

This paper presents the results of investigations of a hitherto unreported disease of corn and of an organism capable of producing it.

The most distinguishing symptom of this disease is the presence of blackened vascular bundles in the stalks and sometimes in the leaves. Associated with the disease to a notable extent are the following abnormalities: Excessive sucker production, prolific stalks, manifestations of which are a tendency for ear development at many nodes or multiple-ear production at one node; a certain type of reddening or purpling of the leaves and stalks; stalks with aborted ears (barren); and stalks bearing nubbins only.

The first part of this paper deals with this group of symptoms in a general way without special regard to causes. The data presented in this connection are based mainly on leaf and stalk color manifestations. However, it was noted that the other characteristics of this group were closely associated.

The data on economic importance show that high percentages of diseased stalks (46.6 per cent in the untreated seed and 42.7 per cent in the treated seed), result from planting ears selected from purple stalks and that yields are reduced. The yield data are presented in two ways. The first shows the average reduction in yield of purple-stalked plants (33.4 per cent with untreated seed and 20.6 per cent with treated seed). This

might be used in estimating loss whenever survey data are at hand. The second shows the reduction in yield due to selecting seed ears from purple-stalked plants (19.7 per cent), and, therefore, the advisability of avoiding selection of seed ears from stalks exhibiting this symptom.

Cephalosporium acremonium has been found closely associated with this group of symptoms and has produced many of these symptoms by pure culture inoculations. Another symptom brought out more strongly by artificial inoculations is an increase in suckering.

The disease caused by this organism is seed-borne and vascular. Therefore, when experimenting with this particular organism, the blackened fibro-vascular bundles were considered the most distinguishing characteristic. Purpling, prolific stalks, barren stalks, nubbin ears, and suckering were noted as being closely associated.

The organism enters the seed through the vascular system and lives within it until placed under conditions favorable for germination. Generally it does not inhibit germination or affect the early vigor. It specifically affects grain production and a general blighting sets in near the end of the growing season.

In one inoculation experiment comprising more than 3,000 plants of Yellow Dent corn and designed to give reliable yield data, the half inoculated with a pure culture of *Cephalosporium acremonium* produced 11.4 per cent less in total yield and 20.0 per cent less in marketable corn than the other half used as a control. The inoculated corn showed also increases of 144.8 per cent in number of suckers, 55 per cent in purple stalks, 114.3 per cent in prolific stalks, 42.4 per cent in nubbin ears, and 55.1 per cent in barren stalks.

Inoculations of a number of strains of Yellow Dent corn have shown different degrees of susceptibility. Even very susceptible strains of this corn may react differently to invasions of this organism as reflected in their outward characteristics.

To reduce losses from this disease it is well to avoid selection of seed ears from stalks having any of this group of symptoms. Probably the best measure of control will come with the development of resistant strains of corn within the varieties. Preliminary seed treatment experiments, not yet ready to be reported in this paper, offer promise of control of this disease.

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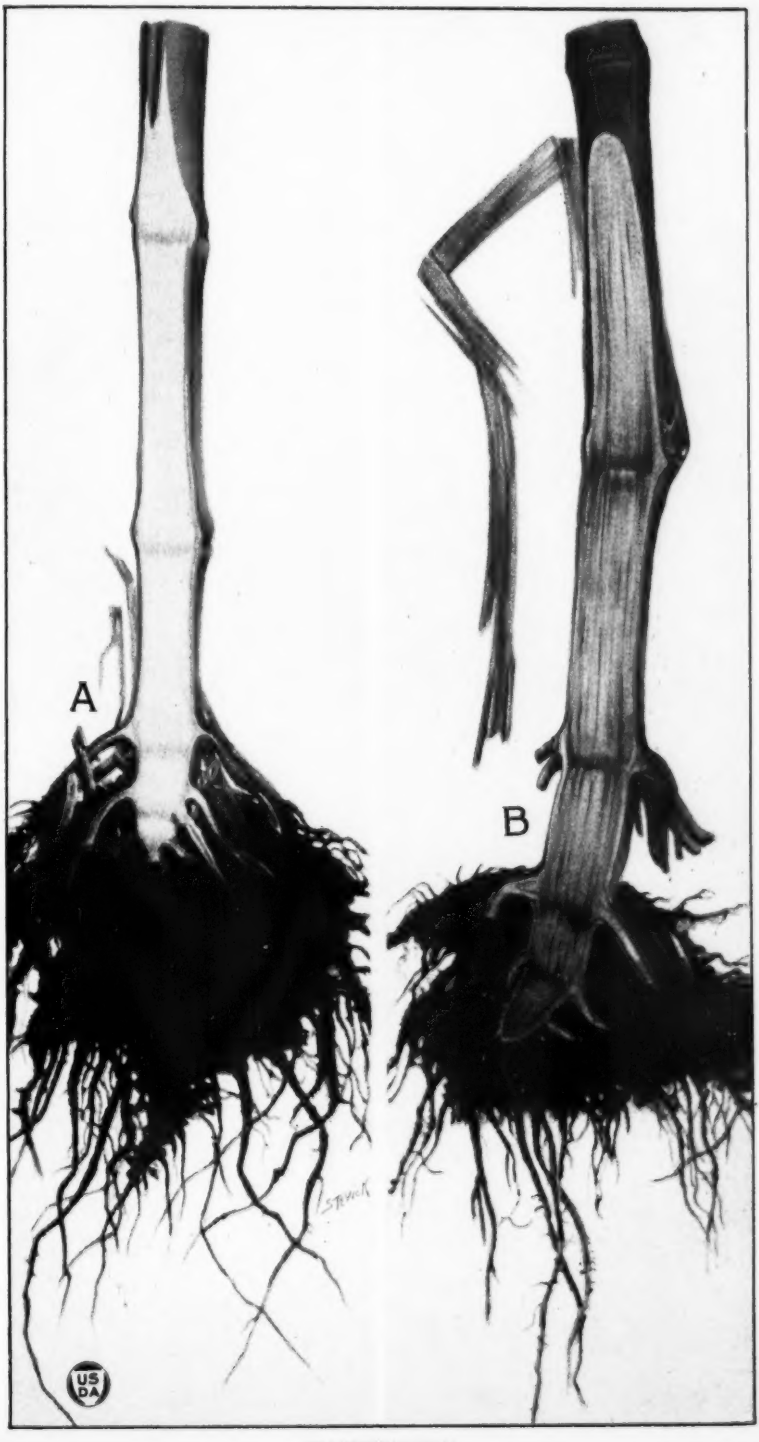
PLATE 1

Bases of Yellow Dent corn plants at time of harvest cut to show internal condition. Healthy plant at left; plant at right infected with *C. acremonium*, showing characteristic blackened bundles and purple stalk.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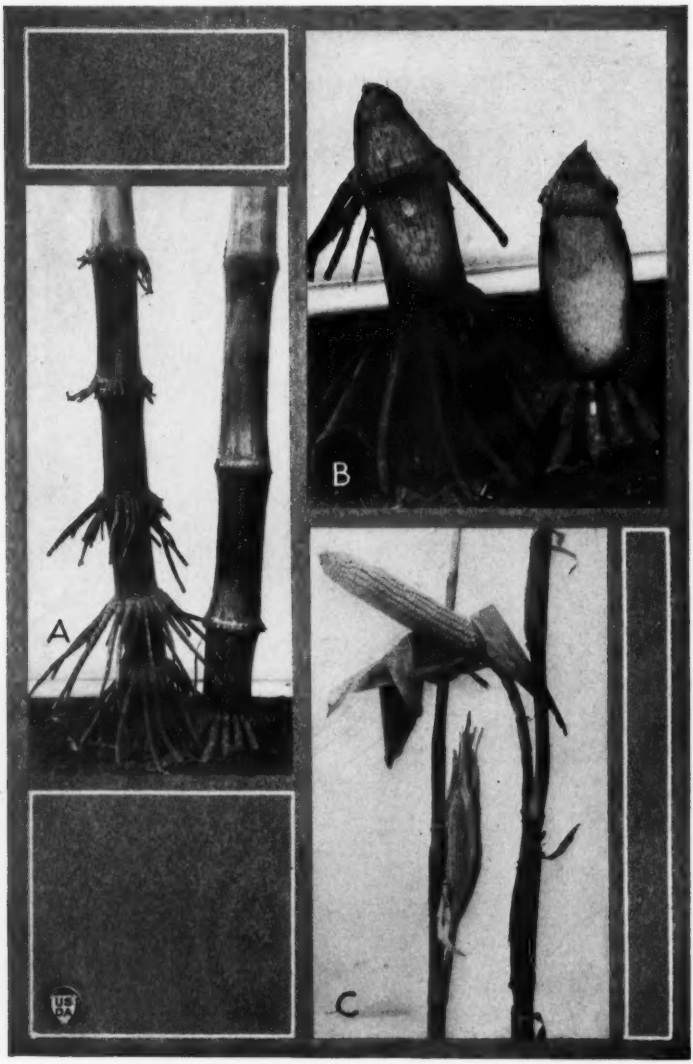
A
B
C

PLATE 2

Portions of diseased (left) and healthy (right) Yellow Dent corn plants in the same hill, grown at Bloomington, Ill., 1918.

A.—Basal portions showing excessive brace roots on diseased stalk (left).

B.—Oblique sections through the basal portions of same stalks. The vascular bundles of the diseased plant (left) are black, while those of the healthy plant are normal in color.

C.—The ear bearing portions of the same two stalks. Diseased stalk (left) with aborted ear; healthy stalk (right) with healthy ear.

PLATE 3

A.—Portion of a main stalk of Funk Ninety-Day corn (*Zea mays indentata*) showing one type of barrenness. The cob is of normal length but the grain production is practically nothing. The leaves were purpled and many of the vascular bundles in the stalk were almost black. Bloomington, Ill., 1920.

B.—Portion of a main stalk of corn (*Zea mays indentata*) showing an extreme case of the most common type of barrenness found in connection with the black-bundle disease of corn. The ear is almost entirely aborted. In other typical cases small nubbins of various lengths are produced which usually curve toward the stalk. Bloomington, Ill., 1920.

C.—Portion of a main stalk of corn (*Zea mays indentata*) showing an attempt at multiple ear production. A high percentage of such plants have blackened vascular bundles in the stalks. Bloomington, Ill., 1920.

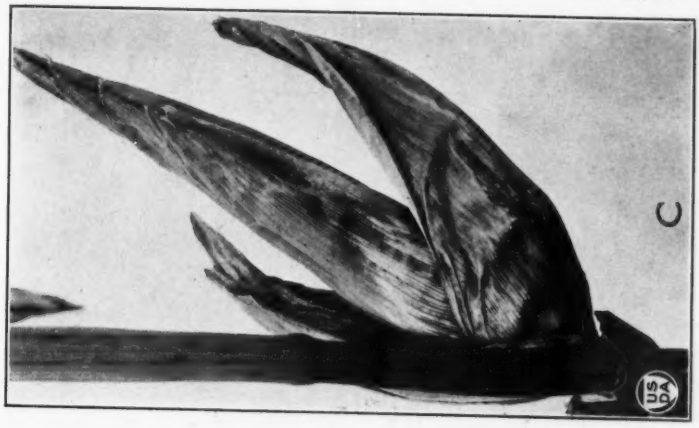
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PLATE 4

Ear rows of Yellow Dent corn planted early on brown silt loam soil of high fertility, near Bloomington, Ill., and photographed in August, 1922.

A.—Grown from nearly disease-free seed. Note absence of excessive suckering.

B.—Grown from the same strain of corn as in A but naturally infected with the fungus, *C. acremonium*. Note excessive suckering.

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PLATE 5

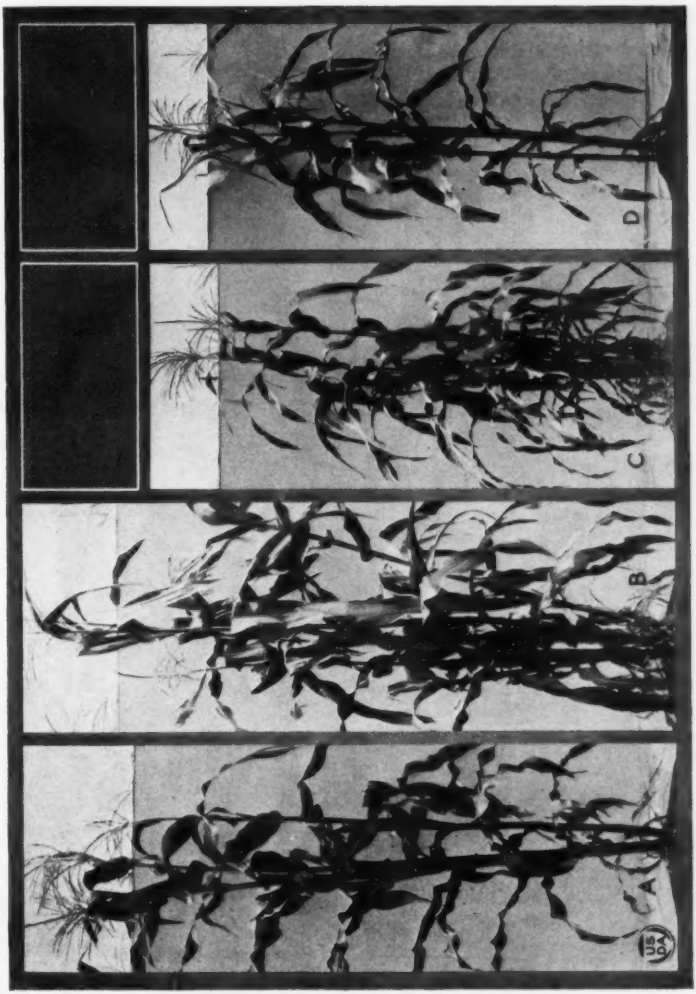
Hills of Yellow Dent corn, uninoculated and inoculated with *C. acremonium*. Bloomington, Ill., 1922.

A.—Hill of corn in the uninoculated control plat grown from nearly disease-free seed. Note the absence of suckering, together with normal formation of ears.

B.—Hill of corn in the inoculation plat (inoculated with *C. acremonium*) grown from nearly disease-free seed. Note the number of suckers.

C.—Hill of corn from nearly disease-free, horny seed; plants inoculated with *C. acremonium*. Note normal development of stalks and ear shoots but an excessive number of suckers.

D.—Hill of corn from weak, starchy seed; plants inoculated with *C. acremonium*. Note spindly stalks without suckers or ear shoots.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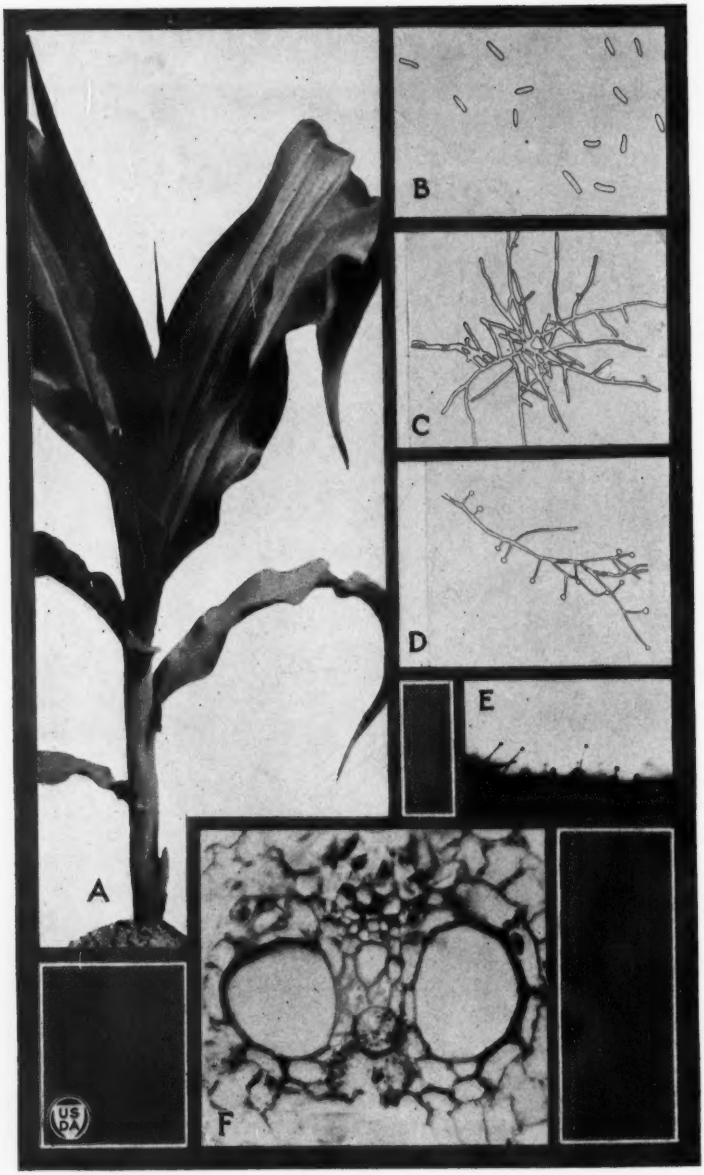
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PLATE 6

A.—Portion of corn plant about three weeks old showing stripes in the leaves caused by natural infection of some of the bundles with *C. acremonium*.

B.—Spores of *C. acremonium*. (× 1000)

C.—Spore of *C. acremonium* 44 hours after germination on potato dextrose agar. (× 250)

D.—Piece of mycelium of *C. acremonium*. (× 200)

E.—*Cephalosporium acremonium* as it appears on the funiculus of germinating kernels of corn when mounted under the low power of the compound microscope. (× 200)

F.—Photomicrograph of a cross section of a blackened vascular bundle in a green leaf of a young dent corn plant. The mycelium of *C. acremonium* is shown almost completely filling the spiral and the annular vessel and occasional strands are shown in the small pitted vessels between the two large pitted vessels.

CHANGES IN HYDROGEN-ION CONCENTRATION PRODUCED BY GROWING SEEDLINGS IN ACID SOLUTIONS[1]

By JEHIEL DAVIDSON, *Associate Chemist*, and EDGAR T. WHERRY, *Chemist in Charge*, *Crop Chemistry Laboratory, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

The importance of the reaction (the active acidity or alkalinity) of the medium in plant life, as well as in biological processes in general, has long been recognized. Studies in this field, however, have gained an impetus with the introduction of improved and delicate methods for estimating hydrogen-ion concentration.

Taking advantage of the ease of manipulation which characterizes these methods, agricultural investigators and botanists have secured a great many data on the relation between plants and the reaction of the medium in which they grow. Much of the recent work, undertaken for the purpose of establishing fundamental principles, has been conducted with nutrient solutions of known composition and with seedlings under conditions involving the least interference from unknown disturbing factors.

Most of the investigations thus far reported have dealt primarily with the effect of the reaction of the medium on plant growth, their ultimate goal being the determination of the specific optimum reaction of the medium for every cultivated plant. Only a few investigators have attacked the problem from the opposite side, studying the effect of the plant on the reaction of the growth medium. This side of the problem, however, is of the highest importance, as it may throw light on the causes of soil acidity under natural conditions, a knowledge of which will greatly facilitate the study of the means of controlling the reaction of the soil under cultivated plants. The work here reported was designed primarily to study the effect of plant growth on the medium.

PREVIOUS WORK

Only a few investigators whose work has a direct bearing on the subject matter of this article will be quoted here.

In 1904 Veitch (*11*) [2] reported the effect of several kinds of plants on the reaction of the soil. In six years oats followed by buckwheat decreased the "total apparent acidity," as determined by the Veitch method (*10*), of nearly all the soils, some of the soils originally acid giving an alkaline reaction. On the other hand, beans followed by

[1] Accepted for publication Nov. 1, 1923. Presented in abstract at the meeting of the American Chemical Society in New Haven, April 6, 1923.

[2] Reference by italic numbers is to the literature cited on page 217.

buckwheat during the same period increased the "total apparent acidity" of the soils used.

Breazeale and Le Clerc (*3*) found that growing wheat seedlings made a sodium nitrate solution alkaline, while solutions of potassium chlorid and potassium sulphate became acid under the same conditions.

Of the investigators using the more recent methods for determining active acidity and alkalinity, Jones and Shive (*7*) studied the effect of wheat seedlings on the hydrogen-ion concentration of a number of nutrient solutions. Wheat seedlings, after having been grown for five weeks in "Shive's Best" solution, were transferred to the nutrient solutions. The initial hydrogen-ion values of these solutions were recorded, as well as the changes resulting after the seedlings had been in contact with the nutrient for 24 and 52 hours. Invariably the changes were in the direction of decreased acidity. The initial reactions of most of the solutions ranged from P_H 4 to P_H 4.8. At the end of the experiment the reactions ranged from 5.3 to 6.1. Two of the solutions the initial reaction of which had been almost neutral remained practically unchanged under the same conditions.

Hoagland (*6*), working with barley and several varieties of beans, found that "in every instance, without an exception, nutrient solutions with an initial acid reaction reached approximately neutral reactions, varying from 6.1 to 7.2, after contact with the plant roots for varying periods of time."

Conner and Sears (*4*), while studying the effect of aluminum salts on plant growth, also found that nutrient solutions with an initial acid reaction tend to become less acid in contact with growing rye, barley, and popcorn seedlings.

Arrhenius (*1*, *p. 81*) reported that plant roots change the reaction of the medium, but that the direction of the change depends on the plant used. Rye brought nutrient solutions of original P_H values 3 and 9 to P_H 5.8; peas brought nutrient solutions of original P_H values 3 and 8 to 4.5; and corn brought nutrient solutions of original P_H values 3 and 7.5 to 6.5.

Later the same author (*2*) studied rice in like manner. Acid reactions in the nutrient solution used were obtained by adding hydrochloric acid and alkaline reactions by adding sodium hydroxide. In all cases the growth of the rice plant brought the reaction to P_H 6.2.

On the other hand, Olsen (*8*) reports that the kind of plant used does not affect the direction in which change of reaction takes place. He concluded that the direction depends primarily on the source of nitrogen in the nutrient solutions. When ammonium chlorid or nitrate is used, all 12 plant species tried caused the solutions to become more acid, although some plants produced the change more rapidly than others. When sodium nitrate was used, however, the solutions uniformly became more alkaline.

Results obtained in the Bureau of Chemistry (Table I) show that factors other than those given by Arrhenius and Olsen (the kind of plants and source of nitrogen) affect the direction of the change in reactions produced in nutrient solutions under the influence of growing seedlings.

TABLE I.—*Changes produced by wheat seedlings in hydrogen-ion concentration of a nutrient solution as affected by its concentration and its initial reaction*[a]

Concentration of solution.	Reaction.		
	Initial.	After 1 day.	After 3 days.
	P_H.	P_H.	P_H.
Undiluted	5.5	5.4	5.2
Do	5.1	4.8	4.6
Diluted 5 times	5.5	5.2	4.1
Do	5.1	3.9	3.6

[a] The acidity data are reported in this and the other tables in the P_H system, not because this system is believed to be the best for the purpose, but because other workers are using it and its use facilitates comparison of these results with theirs.

The nutrient solution used had the following composition:

	Gm.
Calcium nitrate	2.7
Di-potassium phosphate	1.5
Magnesium sulphate	.6
Potassium chlorid	.75
Ferrous sulphate	.01

Twenty wheat seedlings were used for each culture. They were about 4 days old when transferred from tap water to the solution. The nutrient solution was used in two concentrations—full strength and diluted five times. The initial reaction of the full-strength solution, expressed in the P_H system, was 5.5. The other initial reactions were produced by the addition of hydrochloric acid. Variations in the changes of reaction were obtained while using the same plant and solutions of the same composition. The changes were clearly affected by the initial concentration of the nutrient solution as well as by its initial reactions. The general tendency toward increasing acidity, it appears, was due to the use of very young seedlings, most of the investigators cited having worked with seedlings of more advanced age than those used here. In its present phase, however, this investigation deals primarily with the causes of which the changes under discussion are the result.

Very little work has been done to determine the causes of the changes in reaction which occur in nutrient solutions under the influence of growing seedlings. Arrhenius (*1*, *2*) assumed that these changes are due to root excretions which are regulatory and adapt the reaction of the medium to the needs of the plant. He did not, however, bring any evidence to support this assumption. His finding that the reaction of the soil in immediate contact with roots is different from its average reaction in the same vicinity does not prove that the change is due to neutralization. Selective absorption, which is the alternative, would explain the observations just as well.

Breazeale and Le Clerc (*3*), and Hoagland (*6*) also, concluded on the basis of chemical analyses that the changes of reaction produced by growing seedlings in the medium of growth are due to selective absorption. There is, however, some question as to the reliability of direct chemical analysis in dealing with the minute quantities involved, for instance, in a change from P_H 5.6 to P_H 6.8, as was the case in Hoagland's experiment.

EXPERIMENTAL WORK

PROCEDURE

An indirect method was used in this investigation. Only two explanations of the changes which take place in media under the influence of growing seedlings seem possible—neutralization by root excreta and selective absorption.

In the case of neutralization, the effect produced by the growing seedlings should be independent of the kind of acid used, as long as the initial hydrogen-ion concentration and the dissociation constants are the same. In the case of selective absorption, however, the effect of the seedlings in decreasing acidity should depend upon the chemical composition of the acid used. For instance, it would seem that, other factors being equal, the rate and absolute quantity of diminution in acidity will be greater in solutions of nitric acid than in solutions of hydrochloric acid, since nitrogen is a more essential element of plant food than chlorin. Accordingly, seedlings were grown in solutions of several acids of definite initial hydrogen-ion concentrations, and the changes in active acidity of these solutions were compared at intervals. It is assumed that the principles which govern hydrogen-ion concentration phenomena are essentially the same in solutions of pure acids and alkalies as in more complex nutrient solutions with an acid or alkaline reaction.

Only distilled water solutions were employed, as the use of nutrient solutions with the acids would have led to complications likely to interfere with a clear interpretation of the results. For instance, Conner and Sears (*4*) added hydrochloric, nitric, sulphuric, and phosphoric acids, respectively, to a Tottingham solution. As this solution contains phosphates, the addition of a strong acid would naturally cause liberation of phosphoric acid to an extent corresponding to equilibrium relations. Consequently these authors investigated largely phosphoric acid.

Another source of complication resulting from the use of a nutrient solution in this connection would be the change in its composition caused by the feeding activities of the seedlings, with the resulting establishment of new equilibria between the elements. Using the present method, practically only two factors need to be reckoned with—the seedlings and the kind of acids.

PREPARATION OF WATER CULTURES

Wheat seeds were germinated on large perforated aluminum disks floating in tap water, and the seedlings were grown there until they were about 2 inches high. Glasses of approximately 225 cc. capacity containing tap water were covered with paraffined paper perforated with holes smaller than the size of average wheat seeds. The rootlets of the seedlings were then introduced through these holes into the glasses, so that the seeds and plumules rested on the paraffined paper without coming in contact with the liquid media. The seedlings were grown in tap water for about two days to allow good root development and then for a day in distilled water before they were finally transferred to the experimental solutions.

When plants grow in the soil, the seeds are in contact with the same medium as the seedlings, and they may participate in the changes in reaction produced in the medium. In fact, Rudolfs (*9*) found that

ungerminated seeds changed the initial reaction of single salt solutions. As it was the intention to limit this study to the changes produced by the metabolic processes of growing seedlings, however, this procedure, by which the prevention of contact between seeds and the experimental solutions was assured, seemed best. This procedure also made it possible to grow more seedlings in a single culture than the cork method generally used in studies of this nature.

CHANGES OF REACTION IN SOLUTIONS OF VARIOUS ACIDS

The salts of the inorganic acids selected for this experiment are commonly found in soils and are used as fertilizers. The elements which enter into their composition have various functions in plant life and are present in different quantities in plant substance. Their quantitative order is: Nitrogen, phosphorus, sulphur, and chlorin. Functionally, chlorin is considered the least important. A certain number of organic acids were used for the sake of comparison. The experiment was run in duplicate. Samples for analysis were pipetted out through a hole made in the paraffined paper cover. The P_H values were determined colorimetrically. The checks usually were very good. The figures in the tables represent in the majority of cases averages of two determinations. Five seedlings per culture were used in this experiment. The initial reactions of the acid solutions were P_H 4 and P_H 3.

Of the inorganic acids with an initial P_H value of 4, nitric acid decreased most in acidity (Table II). The differences involved were small but consistent, and reappeared when the solutions were renewed. The value of P_H 5, obtained twice in the nitric acid solution, indicates that practically all the acidity attained by the addition of the acid was eliminated, as the untreated distilled water used had an acidity of about P_H 5.3, owing to the presence of carbon dioxide. The results with the inorganic acids of the initial P_H 3 series are inconclusive, owing to the small size of the changes produced, which makes the observations more subject to experimental error.

TABLE II.—*Changes of reaction produced by growing wheat seedlings in solutions of acids*

Acid.	Reaction of solution having an initial P_H of 4.				Reaction of solution having an initial P_H of 3 after 9 days.
	After 1 day.	After 2 days.	After 3 days.	After 10 days.	
	P_H.	P_H.	P_H.	P_H.	P_H.
Hydrochloric	4.50	4.70	[a] 4.65	[b] 4.50	3.0
Nitric	4.70	5.00	[a] 4.90	[b] 5.00	3.1
Sulphuric	4.65	4.70	[a] 4.70	[b] 4.75	3.1
Phosphoric	4.60	4.80	[a] 4.80	[b] 4.70	3.1
Formic	4.65	[a] 6.10	[a,c] 6.10	6.40	6.4
Acetic	4.20	4.50	5.55	6.50	3.1
Oxalic	4.80	[a] 6.10	[a,c] 6.10	[b] 6.40	6.4
Succinic	4.00	4.40	4.90	6.40	3.0
Benzoic	4.00	4.20	4.95	6.45	3.0
Phthalic	4.00	4.00	4.60	4.50	3.0

[a] Solution renewed after this reading was obtained. [b] Seven-day contact. [c] One-day contact.

The changes which occurred in the organic-acid solutions may have been due partly or wholly to microbiological activity, as it was impracticable to keep the solutions sterile. However, oxalic and formic acids, which have the highest dissociation constants, were acted upon to a greater extent than the other organic acids, especially those in the P_H 3 series.

EFFECT OF NUMBER OF SEEDLINGS ON CHANGE OF REACTION

In order to bring out more clearly the differences in the behavior of the individual acids, the foregoing experiment was repeated, using a larger number of seedlings. It was run in duplicate and in two series, one with 10 and the other with 20 seedlings per culture, and was confined to the inorganic acids.

The relative behavior of these acids (Table III) was the same as in the previous experiment. In a general way the rate of response was proportional to the number of seedlings with both of the initial reactions.

In the 20-seedling series, with an initial value of P_H 4, the difference in behavior between nitric acid and the other acids appeared only after the solutions had been renewed twice. At this period, however, the difference between the decreased acidity of the nitric-acid solution and that of the other acids was much more pronounced than in the previous experiment.

In the solutions having an initial P_H 3 value, nitric acid was again more affected by the action of the seedlings than the other acids. Numerically the changes in total values and the differences are small, but quantitatively they are larger than those which took place in the solutions with the initial P_H 4 value. Taking the hydrogen-ion concentration of pure water as a unit, as suggested by Wherry and Adams (*12*, *13*), the decrease in acidity, for example, from P_H 3 to 3.1 would constitute a loss of 2,000 units per liter, while the entire decrease in acidity from 4 to 7 would be only 1,000 units. The fact that losses in acidity designated by the same P_H numerals vary in actual magnitude, depending upon the P_H range in which they occurred, is not always realized. Thus, Conner and Sears (*4*) believed that greater decreases in acidity were produced by growing seedlings in solutions of phosphoric acid with an initial hydrogen-ion concentration of P_H 3.9 and 4.2, which were reduced to 6.3 and 6.4, than in solutions of the same acid with an initial hydrogen-ion concentration of P_H 3.2 and 3.6, which came down to 3.5 and 4.1. Actually, however, the case is just the reverse. Figured on the same basis, the first two transformations involve losses of 1,245 and 625 units, while the last transformations involve losses of 3,150 and 1,700 units.

Nevertheless, it is significant that, while in the solutions of the lower hydrogen-ion concentrations in these experiments, as well as in those of Conner and Sears (*4*), the acidity originally present was practically exhausted, in the case of the higher concentrations the action of the seedlings seemed to have stopped while appreciable amounts of acid were still left in the solutions. But the seedlings also lost their power to reduce acidity in the solutions of the lower initial hydrogen-ion concentrations of these experiments when they were renewed several times. Evidently there are certain limits to the absolute quantities of acid upon which seedlings can act. Within these limits, however, nitric acid was more subject than the other acids to that action of the seedlings which is responsible for decreasing acidity.

TABLE III.—*Effect of number of wheat seedlings on change of reaction produced in inorganic acid solutions*

Acid.	Initial P_H.	Reaction with 20 seedlings.						Reaction with 10 seedlings.					
		After 1 day.	After 2 days.	After 4 days (2-day contact).[a]	After 5 days (1-day contact).	After 6 days (2-day contact).	After 10 days (1-day contact).	After 1 day.	After 2 days.	After 4 days.	After 5 days (1-day contact).	After 6 days (2-day contact).	After 10 days (6-day contact).
Hydrochloric	4.0	4.9	[b]5.05	[b]4.7	4.0	4.0	4.4	4.7	4.8	[b]4.9	4.0	4.0	4.3
Nitric	4.0	4.9	[b]5.15	[b]5.15	4.9	[b]5.0	4.4	4.7	4.9	[b]5.2	4.4	4.4	4.35
Sulphuric	4.0	4.9	[b]5.0	[b]4.7	4.2	[b]4.3	4.4	4.7	4.8	[b]4.9	4.2	4.2	4.3
Phosphoric	4.0	5.1	[b]5.1	[b]4.85	4.0	[b]4.3	4.0	4.7	5.0	[b]5.0	4.0	4.1	4.0
Hydrochloric	3.0	3.0	3.0	3.0	3.1	3.2	3.1	.....	.....	.....	.....	.....	3.0
Nitric	3.0	3.0	3.0	3.1	3.2	3.2	3.3	.....	.....	.....	.....	.....	3.2
Sulphuric	3.0	3.0	3.0	3.1	3.2	3.2	3.2	.....	.....	.....	.....	.....	3.0
Phosphoric	3.0	3.1	3.1	3.1	3.2	3.2	3.2	.....	.....	.....	.....	.....	3.0

[a] Specified days of contact refer to P_H 4 series only.

[b] Solution renewed after this reading was obtained.

EFFECT OF PREVIOUS TREATMENT OF SEEDLINGS ON CHANGES IN REACTION OF ACID SOLUTION

The behavior of seedlings which had been grown previously in a complete nutrient solution, as compared with that of seedlings which had previously been grown in incomplete nutrient solutions, defective in chlorin, nitrogen, sulphur, and phosphorus, was studied. For instance, the seedlings which were to be grown in a solution of hydrochloric acid were first grown in a nutrient solution which was lacking in chlorin.

The nutrient solution used was diluted five times. The defective solutions were made up so that they differed as little as possible from the complete solution, except for the missing element. The seedlings were divided into two series: One series was grown for five days in the complete nutrient solution, and the other in the defective solutions. All seedlings were then grown for a day in distilled water and finally transferred to the acid solutions with an initial reaction of P_H 4. After the changes for two successive days had been recorded the acid solutions were renewed and the changes in P_H values were again recorded after a one-day and after a three-day contact. All seedlings were then transferred to the complete nutrient solutions diluted 10 times. Here they grew for two days, after which they were transferred to the complete and defective nutrient solutions, diluted 10 times. Two days later they were transferred to the acid solution with the initial reaction of P_H 3.6, in which they were kept for three days, when the changes in reaction were again recorded.

TABLE IV.—*Effect of complete and incomplete nutrient solutions in which seedlings had been previously grown on the changes produced in inorganic acid solutions* [a]

Acid.	Previous condition of solution.	Reaction.				
		After 1 day.	After 2 days. [b]	After 3 days [c] (1-day contact).	After 5 days (3-day contact).	After 12 days (3-day contact).
		P_H.	P_H.	P_H.	P_H.	P_H.
Hydrochloric	Complete	4.65	4.90	4.45	4.85	4.25
	Complete—Cl	4.60	4.70	4.50	4.65	3.95
Nitric	Complete	4.60	5.10	4.50	4.85	3.95
	Complete—N	4.55	4.70	4.60	4.70	3.85
Sulphuric	Complete	4.65	5.40	4.45	4.85	4.00
	Complete—S	4.65	4.95	4.50	4.65	3.90
Phosphoric	Complete	4.70	5.50	4.60	5.15	5.20
	Complete—P	4.60	4.80	4.65	4.70	4.00

[a] Initial P_H 4.0; number of seedlings, 15.
[b] Solutions renewed after these readings were obtained.
[c] All seedlings were held in complete (1:10) solution for 2 days, then for 2 days in complete and defective (1:10), and were then transferred back to acid solutions with an initial reaction of P_H 3.6.

The results (Table IV) show that the previous growth of the seedlings in a complete nutrient solution did not affect their activity, so far as their ability to lower the initial hydrogen-ion concentration was concerned. The previous growth of the seedlings in defective solutions, for the purpose of creating an avidity for the elements of the acids with which they were subsequently to be brought in contact, resulted, contrary to expectation, in a depressed activity with reference to changes

in reaction of the acid solutions. This is shown by the three final readings (before solutions were renewed or discarded) which give consistently higher acidities for the seedlings grown previously in defective nutrient solutions. The defectiveness of the previous nutrient solution evidently created in the seedlings some functional disturbance either general or with reference to their behavior toward the elements lacking.

The most significant result obtained in this experiment, however, is that in the case of the older seedlings nitric acid had ceased to be affected by the action of the seedlings to a greater extent than the other acids. Its place was taken by phosphoric acid, as was shown by the three final readings in the series which had been grown in the complete nutrient solution.

The initial acidity of phosphoric acid solution was reduced from P_H 3.6 to P_H 5.2 in the final reading, a reduction which is strikingly greater than any obtained in the case of the other acid solutions. The differences in the behavior of the various acids were thus brought out more distinctly. The preference of the seedling for the different acids was also shown to vary with the conditions and with the stage of growth.

EFFECT OF AGE OF SEEDLINGS ON CHANGES IN REACTION IN ACID SOLUTIONS

The effect of the age of seedlings on their behavior toward nitric and phosphoric acids, which was unexpectedly brought out by the previous results, was studied further.

The usual procedure was followed. After the seedlings had been transferred to glasses and grown in tap water for three days, the roots were kept in contact with distilled water for two hours. The seedlings were then divided into two series. The seedlings of the first series were transferred to the acid solutions immediately. The seedlings of the second series were transferred to the acid solutions when 15 days old, after having been grown for 10 days in the nutrient solution diluted five times. The initial reaction of the acid solutions used was P_H 3.6, which was more suitable than the initial reaction of P_H 4, as the initial acidity was thereby increased 250 per cent and the changes produced were more pronounced.

TABLE V.—*Effect of age of seedlings on changes produced in inorganic acid solutions*[a]

Acid.	Initial age of seedlings.	Reaction. After 1 day.	After 2 days.[b]	After 4 days (2-day contact).	After 6 days (4-day contact).	After 11 days (1-day contact).[b]	After 13 days (2-day contact).[c]	After 17 days (3-day contact).	After 20 days (5-day contact).
	Days.	P_H.	P_H.	P_H.	P_H.	P_H.	P_H.	P_H.	P_H.
Hydrochloric	5	4.05	4.00	3.6	3.70	.....	.....	.....	.....
Nitric[d]	5	4.40	5.10	4.3	4.70	.....	.....	.....	.....
Sulphuric	5	3.90	4.10	3.6	3.65	.....	.....	.....	.....
Phosphoric	5	4.15	4.45	3.9	3.95	.....	.....	.....	.....
Hydrochloric	15	.....	.....	.....	.....	4.80	3.90	4.00	4.35
Nitric	15	.....	.....	.....	.....	4.75	4.00	4.20	4.60
Sulphuric	15	.....	.....	.....	.....	4.90	3.90	4.30	4.50
Phosphoric	15	.....	.....	.....	.....	5.20	4.20	4.45	6.15

[a] Initial P_H, 3.6; number of seedlings, 20.
[b] Solution renewed after these readings were obtained.
[c] Transferred to complete nutrient solution (1:5) for 2 days and then back to acid solutions.
[d] Initial P_H, 3.5.

The results (Table V) show again and more clearly that the preference of the seedlings at an early stage of growth is for nitric acid and at a later stage for phosphoric acid. The age factor in this experiment also includes the factor of previous mineral nutrition. As a rule, however, the two are inseparable, as age in the sense of advanced stage of growth can not be measured merely by the number of days which elapse from the time of germination.

DISCUSSION OF EXPERIMENTAL RESULTS

The fact that the activity of seedlings in decreasing the initial acidity of the medium is preferential, resulting in the greatest decrease in acidity in one acid at one set of conditions and in another acid at another set of conditions, indicates that the cause of these changes is preferential absorption by the plants.

The term "preferential absorption" seems more appropriate here than the commonly used term "selective absorption." "Selective absorption" might imply that some substances are excluded, while "preferential absorption" indicates merely a higher rate of absorption of one substance as compared with that of another.

If the changes in the initial reaction of the acid solutions were a result of neutralization by secreta from the roots, they would be expected to be controlled by the active acidity factor only, and different acids with the same initial hydrogen-ion concentration and the same dissociation constants would behave alike. If the preferential action of the plants were in favor of nitric acid throughout, reduction by microorganisms might have suggested itself. It has been shown elsewhere (*4*), however, that the possibility of nitrate reduction is eliminated under the conditions of these experiments. But the fact that the preference of the older seedlings is for phosphoric acid lends additional strength to the absorption theory, which is further supported by the fact that in plant life nitrogen and phosphorus are, at least quantitatively, the most essential of the acid-forming elements dealt with in this investigation.

SUMMARY

Wheat seedlings were grown in solutions of hydrochloric, nitric, sulphuric, phosphoric, formic, acetic, oxalic, succinic, benzoic, and phthalic acids. The changes in reaction produced by their growth, recorded at certain intervals, show that:

Of the inorganic acids, the greatest changes were produced in nitric acid at early stages of growth of the seedlings and in phosphoric acid at later stages. Phosphorus and nitrogen being the most essential elements of plant growth contained in the acids used, it may be concluded that the changes in initial reaction produced by plants in the medium in which they grow are due to absorption rather than to neutralization.

The previous growth of the experimental seedlings in nutrient solutions deficient in acid-forming elements diminished their ability to decrease the acidity of the acid solutions. Apparently the deficiency of the previous nutrient solutions produced functional disturbances in the seedlings.

The greatest changes from the initial reactions were produced in the solutions of the organic acids. This, however, may have been due partly or wholly to microbiological activity and needs to be studied further.

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THE NUTRITIVE PROPERTIES OF WILD RICE (*ZIZANIA AQUATICA*)[1]

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INTRODUCTION

Wild rice, a cereal which is distantly related to the cultivated rice, is an annual grass growing in shallow lakes and sluggish streams over a widely extended territory in North America. It is used rather extensively as a food by some of the Indian tribes of the upper Mississippi Valley and, to a more limited extent, in hotels and in private homes during the game seasons. It also serves as an important source of food for wild water fowl.

Eight years ago the Division of Agricultural Biochemistry undertook an investigation of wild rice, both because the cereal grows quite extensively in Minnesota and because it was desirable to ascertain its food value in order to determine whether it was advisable to improve the methods of its cultivation, harvesting, curing, and marketing, so that it could become of agricultural importance. At that time, as at the present, practically no means were employed to preserve the natural rice beds or to plant the rice in new localities, and the methods of harvesting and curing were very crude and wasteful.

Wild rice usually matures in the latter part of August or in September after the first frost. Shortly before this time the Indian harvesters go into the rice beds and tie the standing stalks into small bunches; then, when the grain is sufficiently matured, they return in their canoes and, holding the bunches over their boats, knock the ripened kernels into the bottoms of the boats. Only about 50 per cent of the ripe grains fall into the boats, the rest being lost in the mud of the lake.

Since the rice is gathered before it is fully ripened, it must be cured or artificially ripened. This process also aids in removing the tenacious hull. The grain is cured, (1) by the sun, (2) by smoke and heat from a slow fire under the rice, which is spread on a framework above, and (3) by parching or popping in a metal vessel. After drying, the hulls are threshed off either by treading or by striking with paddles. The hulls are finally blown off by the wind or with a fan. Such is the primitive method used by both the Indian and the white man to gather and cure the rice.[2]

CHEMICAL ANALYSIS OF WILD RICE

When this investigation was begun, in 1915, the biological method for the analysis of a foodstuff was unknown. Later, when this method of appraising the value of foods had developed, the investigation was

[1] Accepted for publication July 11, 1923. Published with the approval of the Director as paper No. 398, Journal Series, Minnesota Agricultural Experiment Station.

[2] A general description of wild rice, its habitat, production, etc., is given in JENKS, A. E., THE WILD RICE GATHERERS OF THE UPPER LAKES. In 19th Ann. Rpt. Bur. Amer. Ethnol. (1897–98), pt. 2, p. 1013–1137, illus., 1900. A brief account of the plant, its culture, etc., is found in CHAMBLISS, C. E., WILD RICE. U. S. Dept. Agr. Circ. 229, 16 p., illus., 1922.

expanded to include a biological analysis of the wild rice. The first part of the investigation was therefore confined to the chemical composition of the cereal. Four samples of rice were secured for analysis, two being obtained in the local markets and two direct from the Indians in the region of the Minnesota rice lakes. Three of these samples were of quite uniform appearance, consisting of slender cylindrical kernels varying from one-half inch to nearly one inch in length, and were of a dark slate color. The remaining sample (No. 2) was quite different in its physical appearance, the kernels being very long and thick, and many of the grains being parched to such a degree that they resembled puffed cereals.

Table I shows the results of the analysis of the four samples of wild rice, and, for comparison, an analysis of a polished and an unpolished cultivated rice. It is seen that the proportions of protein and soluble carbohydrates in the wild rice are considerably greater than in either sample of the cultivated variety. The soluble carbohydrates of the rice are probably formed in large measure during the parching process which is commonly used in removing the hulls. The ether extract of the wild variety is approximately double that of the polished cultivated rice, but considerably less than that of the unpolished cultivated variety. Much of the embryo is doubtless removed in the wild rice by the parching process to which kernels are subjected in order to remove the hulls, but, since the parching process is not at all uniform, the embryo is not entirely removed, as it is in the polishing of the cultivated rice.

The amount of inorganic material is considerably higher in the case of the wild rice than in that of the cultivated rice. Table II shows the distribution of the inorganic elements in the ash of the two varieties. For comparison, an analysis of dried skim milk is also included in this table.

TABLE I.—*Comparison of chemical composition of wild rice and cultivated rice*

Sample No.	Moisture.	Ash.	Protein.	Ether extract.	Fiber.	Starch.	Soluble carbohydrates as dextrose.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	7.74	1.09	13.36	0.455	1.39	65.26	2.98
2	7.85	1.38	13.97	.893	1.41	61.69	3.69
3	8.93	1.17	14.62	.718	1.94	60.47	2.33
4	7.83	1.25	14.40	.658	1.29	62.03	2.93
Cultivated unpolished rice	12.22	1.01	5.04	2.01	1.08	69.50	.85
Cultivated polished rice	12.30	.40	8.0	.30	.20	79.00	.....

TABLE II.—*Distribution of inorganic elements in wild and cultivated rice in comparison with dry skim milk*

Substances compared.	Ca.	Mg.	K.	Na.	P.	S.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Wild rice	0.018	0.080	0.055	0.064	0.424	0.252
Cultivated polished rice	0.008	0.027	0.069	0.021	0.102	0.105
Dry skim milk	1.33	0.147	1.27	0.488	0.979	0.357

As the composition of the inorganic matter of milk is adequate to meet the needs of the growing animal, it is readily seen that both varieties of rice have very inadequate amounts of the essential ash constituents. This point will be discussed further in connection with the biological analysis of the rice.

BIOLOGICAL ANALYSIS OF WILD RICE

Since it is now recognized that a chemical analysis of a foodstuff is very inadequate for judging its food value, the investigation of the wild rice also included a biological analysis. From this analysis it is possible to compare the value of this cereal with that of many of the cereals in common use, and thus evaluate it as a food product.

The method of procedure was that commonly used when it is desired to determine the deficiencies of a single foodstuff. Rice was fed, with no additions; and when it became evident that growth could not be induced on such a food, additions of known purified food constituents were made singly and in groups until a food was produced which gave satisfactory growth. The results of this procedure make it clear that the dietary properties of wild rice are much like those of our common cereals, but somewhat better than those of polished rice.

The wild rice used in the feeding experiments was obtained in the local market, ground to a fine meal, and fed, both unmixed and with certain additions, to young rats weighing from 70 to 80 gm. The rats were kept on sawdust bedding in clean, well-ventilated cages. The efficiency of these different rice rations in promoting growth in the rat is set forth in figures 1 and 2.

The ration of the rats of Lot 1 (fig. 1) consisted of wild rice with no additions. This proved to be an entirely inadequate food, for the greater number of animals in this lot constantly lost weight, and by the end of the third week were in an extremely emaciated condition.

The first addition that it seemed advisable to make was that of inorganic constituents, since the chemical analysis of the rice had shown it to be very deficient in the mineral elements essential for growth. As the ash analysis of the wild rice (Table II) did not differ greatly from that of polished rice, the same salt mixture that McCollum and Davis [3] used with polished rice was added to the cereal. This salt mixture consisted of sodium chlorid 5 parts, dipotassium phosphate 12.1 parts, monocalcium phosphate 2.56 parts, calcium lactate 29.44 parts, and ferric citrate 1 part.

The ration of the rats of Lot 2 (fig. 1) contained 4 parts of this salt mixture and 96 parts of wild rice. Although the addition to the inorganic content of the ration greatly improved the growth of the rats, this growth was still far from normal. It is interesting to compare these growth curves with those obtained by McCollum and Davis [4] on a ration consisting of polished rice with the addition of the same salt mixture in the same proportion. Their rats not only failed to respond to this addition but steadily lost weight, thus indicating that the cultivated polished rice is more deficient in necessary food constituents than the wild variety.

[3] McCollum, E. V., and Davis, M. The nature of the dietary deficiencies of rice. *In* Jour. Biol. Chem., v. 23, p. 195. 1915.
[4] McCollum, E. V., and Davis, M., op. cit., p. 195.

As it was evident from the growth curves of this lot that the rice-salt diet was inadequate to promote normal growth, vitamin A was added, for the reason that cereals as a class are deficient in this factor. A ration consisting of wild rice 91 parts, salt mixture 4 parts, and butter fat 5 parts was fed to the rats in Lot 3 (fig. 1). The addition of the butter fat made a great improvement in the rice-salt ration, as almost normal growth was obtained for a period of two months and pregnancy was secured twice in the case of one individual. However, the rats of the first litter were all born dead, and in the second pregnancy the rat died before the young were born, autopsy showing nine fetuses at term.

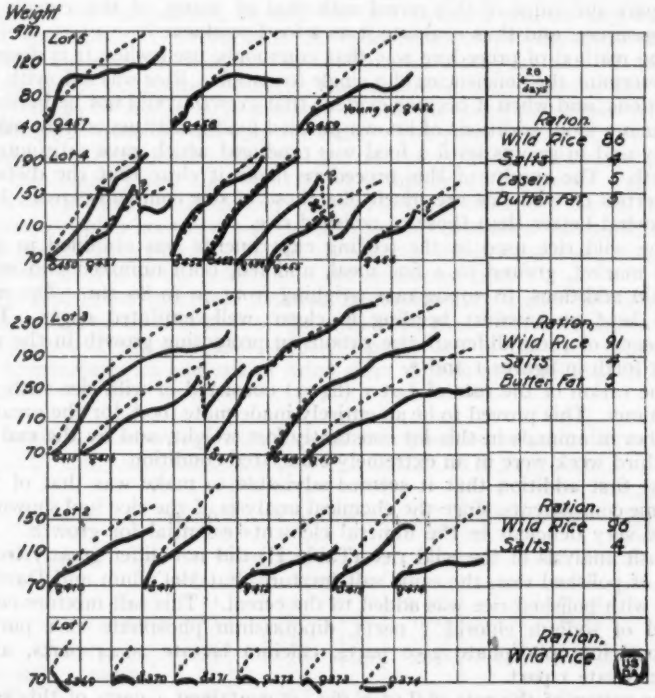

FIG. 1.—Rate of growth of rats on rations consisting of wild rice alone and wild rice with various additions. + indicates death of rat 454. Lot 4; y=young.

This result, together with the fact that none of the growth curves in this group approached the normal after the second month, showed that the ration was still deficient. Nevertheless, comparison of these growth curves with those of McCollum and Davis,[5] rats on a similar ration containing polished rice shows that the wild rice with the addition of proper quantities of salts and butter fat is a much better ration than polished rice with the same additions. The growth curves of Lot 3 indicate that the proteins and the quantity of vitamin B are fairly adequate, because if either one or the other or both were deficient, such good growth could not have been attained.

[5] McCOLLUM, E. V., and DAVIS, M., op. cit., p. 199.

Considering that many of the cereals not only are low in inorganic constituents and vitamin A but also have proteins of poor quality, casein was the next addition made to the rice-salt-vitamin A ration. The new ration, consisting of wild rice 86 parts, salt mixture 4 parts, butter fat 5 parts, and casein 5 parts, was fed to the rats of Lot 4 (fig. 1). The addition of the casein allowed the rats to grow normally, although reproduction and rearing of young were not satisfactory. Each of the females had one litter, but out of a total of 29 rats in the second generation only 3 could be reared to adult size, the other 26 either being born dead, dying shortly after birth, or being destroyed by the mother.

The three rats of the second generation, Lot 5 (fig. 1), had a total weight of 147 gm. at the end of the fifth week, when they were weaned and given the mother's ration. For the first four weeks their growth was normal, but after this period growth was much below normal. This failure to grow normally was probably due to the fact that the rats had a lung infection which was manifesting itself in our rat colony at the time, although it might have been due to a dietary deficiency which prevented normal development in the second generation.

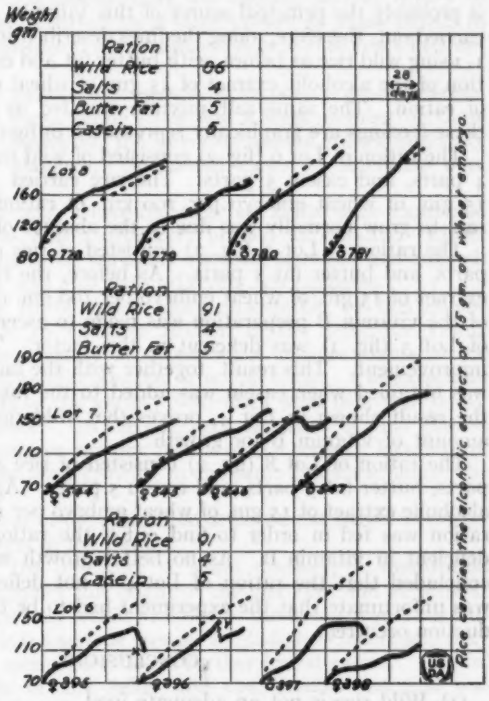

FIG. 1.—Growth curves of rats, illustrating the effect of additions of vitamin B to wild rice rations which had been modified by casein, butter fat, and mineral salts. + indicates death of rat 395, Lot 6; y denotes young.

The excellent results obtained by the addition of the small amount of casein showed that the protein of the wild rice was not wholly adequate for growth, and in fact was probably the only other factor deficient in the cereal. The two additions already made, inorganic salts and vitamin A, with the further addition of protein, make wild rice a food better suited to promote growth than the same additions to polished cultivated rice. Thus, McCollum and Davis [6] found that the addition of 2 per cent of casein to a ration consisting of polished rice, salt mixture, and butter fat did not lead to growth, but that growth was greatly stimulated by the addition of wheat embryo. The growth curves of their rats,[7] whose

[6] McCOLLOM, E. V., and DAVIS, M., OP. CIT., p. 200.
[7] McCOLLUM, E. V., and DAVIS, M., OP. CIT., p. 191, 223.

ration consisted of polished rice 64 parts, casein 13.4 parts, salt mixture 2.4 parts, butter fat 5 parts, agar 2 parts, and dextrin 13.2 parts, carrying the extract of 5 gm. of wheat embryo per 100 gm. of ration, were no better than the growth curves of the rats of Lot 4 of this experiment, whose ration contained but 5 per cent of additional protein and no addition of vitamin B.

Although cereals contain an abundance of vitamin B, it was thought that wild rice might be deficient in this factor because the parching process, often used to remove its hulls, might also char the embryo, which is probably the principal source of this vitamin. A different series was carried out, therefore, along the lines described in connection with Figure 1, using wild rice as before, with butter fat and casein, but with the addition of the alcoholic extract of 15 gm. of wheat embryo to each 100 gm. of ration. The same salt mixture was fed as before. The results of these feedings are graphically represented in figure 2.

The ration of Lot 6 (fig. 2) consisted of wild rice 91 parts, salt mixture 4 parts, and casein 5 parts. The rice carried the alcoholic extract of 15 gm. of wheat embryo per 100 gm. of ration. The failure of these rats to grow normally was due to the absence of vitamin A.

The ration of Lot 7 (fig. 2) consisted of rice 91 parts, salt mixture 4 parts, and butter fat 5 parts. As before, the rice carried the alcoholic extract of 15 gm. of wheat embryo per 100 gm. of ration. This addition of the vitamin B preparation was made to ascertain whether the ration of Lot 3 (fig. 1) was deficient in this factor. The addition caused no improvement. This result, together with the fact that excellent growth was obtained when casein was added to the ration of Lot 3, producing the result shown in Lot 4, proves that wild rice contains an adequate amount of vitamin B for growth.

The ration of Lot 8 (fig. 2) consisted of rice 86 parts, salt mixture 4 parts, butter fat 5 parts, and casein 5 parts. Again the rice carried the alcoholic extract of 15 gm. of wheat embryo per 100 gm. of ration. This ration was fed in order to find out if the ration of Lot 4 (fig. 1) was deficient in vitamin B. As no better growth was obtained, it can be concluded that the ration of Lot 4 is not deficient in this factor. It was unfortunate that the experiment had to be terminated before reproduction occurred.

CONCLUSIONS

(1) Wild rice is not an adequate food.

(2) Although chemical analysis shows a higher percentage of protein present than in many cereals, wild rice resembles many other cereals in containing proteins of rather low biological value. It further resembles other cereals in containing inorganic material unsuitable for the promotion of growth, and in being very deficient in vitamin A, although enough of this vitamin is present to prevent xerophthalmia.

(3) Wild rice has a greater food value than the cultivated polished rice, because its proteins are of better quality and because it contains adequate amounts of vitamin B for animal growth, which is not true of the polished cultivated rice.

A BACTERIAL BLIGHT OF GLADIOLI[1]

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INTRODUCTION

In the summer of 1922 a disease that was causing serious injury to the leaves of gladioli was reported by a large grower of these plants in the Middle West. The disease if present at all in previous years had not attracted attention.

In 1923 in the same fields, and especially in poorly drained areas, the disease appeared on some young stock and a large number of these plants were destroyed. However, the infection abated rather suddenly and caused no further damage. Occasional cases of infection were noted on the older stock.

In early September (1922) when the affected fields were examined by the writer, the leaves had a distinctly burned appearance. Closer observation showed them to be variously spotted, browned and ragged. Many leaves were heavy and stiff with adhering soil particles.

Young stock appears to be much more susceptible to this disease than mature plants. Of 16 varieties studied in the field, the young stock of 15 varieties was severely attacked, while mature plants of the same varieties showed only slight spotting or no infection. One exception was noted, mature plants of the variety Schwaben were nearly as badly infected as the young stock. On the contrary, no trace of this disease was found on either young or mature plants of the variety Mrs. Frank Pendleton, although these were growing in the same fields and under the same general conditions.

Microscopic examination of the discolored areas on the leaves revealed great quantities of bacteria in the tissues.

In a search through the literature of plant diseases, no description has been found that resembles this blight of the gladiolus and at present this mid-west farm is the only locality known to the writer where the disease occurs. But since it is a serious menace to gladiolus culture and may be present elsewhere, it seemed worthy of further investigation. Careful observations were made in the fields and considerable material was collected for study. During the succeeding months experiments on plants in greenhouses and in outdoor beds and studies in the laboratory have established the following facts concerning the disease.

Lesions are first visible as narrow, horizontal, water-soaked spots (Pl. 1, A); the later progress of the infection is mostly in a longitudinal direction so that the spots become more or less regularly formed squares or rectangles (Pl. 1, C and E). Adjoining lesions unite until often the entire leaf from the tip to the base is involved. This widespread infection of the leaf is most noticeable in the young stock. All parts of the leaves of blooming stock are also subject to attack, but the infection is quite

[1] Accepted for publication Dec. 14, 1923.

often found only on the middle area of the leaf. As seen in fresh material, these spots are translucent and watersoaked. In reflected light the color is a very dark green; in transmitted light it is a bright green. The translucency of the spots is permanent, but when old and dry the colors are various shades of brown.

Considerable quantities of a bacterial exudate flow from the affected tissues. In quiet, dry air this exudate forms slender, twisted white columns 2 to 10 mm. long. Under moist conditions it forms a viscid film over the leaf. When dry it may be a thin, brittle layer or it may occur in the form of numerous small droplets. Wind-blown soil, tiny insects, and various small particles become embedded in this exudate, often in sufficient quantities to cover the leaf surface. The exudate seems to be composed entirely of bacteria; it dissolves readily in water and doubtless is a factor in the dissemination of the disease.

The name *Bacterium gummisudans* proposed for the causal organism of this disease, was suggested by the copious exudate from the lesions.

ISOLATION AND REINFECTION

Yellow, viscid bacteria were repeatedly and easily isolated from the lesions and from the exudate, and numerous inoculation experiments have proved these bacteria to be the cause of the disease.

From lesions produced by pure culture inoculations the organism was reisolated on agar-poured plates and with subcultures from single colonies typical reinfections were obtained. Repeated experiments in inoculating, reisolating, and reinfecting leave no doubt either as to the cause of the disease or as to the general character of the lesions.

Young plants grown from cormels were readily infected. Older plants were more resistant. Of six varieties of mature plants used for inoculation experiments, Schwaben was the only one that became generally infected; Mrs. Francis King was very slightly infected. On Mrs. Frank Pendleton no infection occurred. These inoculation results agree with the field observations in regard to relative susceptibility of young and mature plants and of varieties.

Iris, hyacinth, and barley were inoculated but no infections resulted.

MORPHOLOGY

The organism is a short, actively motile rod, occurring singly and in pairs in the host and in most culture media. In beef bouillon it forms long chains. It measures when stained with carbol fuchsin 1 to 2.8μ by 0.6 to 0.8μ; no spores have been seen. Capsules are formed on beef peptone agar and on potato dextrose agar. The single polar flagellum is 3 to 9μ long. The flagella were demonstrated by the use of Casares–Gil's staining method. It is Gram-negative and not acid-fast.

CULTURAL CHARACTERS

On peptone beef [2] agar the colonies are pale yellow (amber yellow [3]), circular, transparent; the surface is smooth and the interior has concentric striations (Pl. 2, F). With age the growth becomes less transparent and the striae disappear. Buried colonies are oval to spindle-shape and have definite margins.

[2] All the beef media used in this study were made from fresh beef infusion with addition of 1 per cent Difco peptone corrected to +14 to +18 Fuller's scale (P_H 6.8 to 6.4).

[3] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

Beef bouillon clouds only moderately but forms a thick, yellow rim and a thin pellicle. The rim and pellicle are extremely viscid. The growth is viscid in most media but especially so in beef infusion peptone media.

Liquefaction of gelatin is not rapid at 18 to 20° C. Colonies on plates showed first signs of liquefaction on the fifth day. In stab cultures the upper fifth of the medium was liquefied by the seventh day. Blood serum becomes translucent and partly liquefied.

On potato cylinders the growth is pale yellow and abundant, usually covering the cylinder and filling the water with a thick growth. The potato is slightly browned.

A slight acidity is produced in milk cultures. A soft, smooth curd forms, but it is rapidly and completely digested. As the casein is digested the medium becomes translucent,but the translucency is obscured by the large quantity of almost opaque, viscid substance that practically fills the medium. Heavy, yellow, viscid rims and yellow sediment develop in milk cultures. Tyrosin crystals are formed.

Litmus in milk is slowly reduced.

No gas is formed from dextrose, saccharose, lactose, maltose, galactose, or glycerin. Slight amounts of acid are produced in cultures containing dextrose and saccharose. With lactose and glycerin the reaction is consistently alkaline.

Growth is scanty in Cohn's and Uschinsky's solutions; scanty and fugitive in Fermi's solution.

Ammonia and hydrogen sulphid are produced in cultures. Several tests for indol have given negative results. Controls of *B. coli* gave positive indol reactions.

Nitrates are not reduced.

Sodium chlorid is not well tolerated, 1 per cent definitely reducing growth, and no growth occurring above a 2 per cent concentration.

The optimum reaction for growth in peptone beef bouillon is +14 to +17 (P_H 6.8 to 6.5).[4] Even under only slightly adverse conditions growth is slow, and no growth occurs if the media is much below or above the optimum. For example, in one of the experiments for the determination of the thermal death point, peptone beef bouillons titrating +6, +10, +15, and +17 (P_H 7.6, 7.2, 6.7, and 6.5) were used. All four were made from the same beef infusion stock. The tubes had the same amount of inoculum, and the heating tests were simultaneous. Cultures in +15 and +17 survived higher temperatures than those in +6 and +10. In the controls growth was retarded in the +6 and +10 and never became so heavy as in the +15 and +17 media. The minimum is at 0 (P_H 8.2), the maximum at +25 (P_H 5.7).

The toleration of organic acids was tested by adding 0.1 and 0.2 per cent, respectively, of citric, malic, and tartaric acids to a neutral bouillon base. The bacteria grew normally in the 0.1 per cent acids (+15, P_H 7.0) and not at all in the 0.2 per cent (+27, P_H 5.7).

TEMPERATURE RELATIONS

In beef bouillon the optimum temperature is near 30° C.; the minimum is below 2°. Growth begins but does not continue at 36°. The thermal death point is near 50°.

[4] QUIRK, Agnes J., and FAWCETT, Edna H. HYDROGEN-ION CONCENTRATION VS. TITRATABLE ACIDITY IN CULTURE MEDIUMS. *In* Jour. Infect. Diseases, v. 33, p. 20. 1923.

DESICCATION

The majority of young cultures dried on cover glasses were dead inside of 24 hours, and all within 3 days.

The bacteria were easily isolated from dry leaves up to 10 days after collection. After 10 days' drying, success in isolation was infrequent. In only 1 case out of 14 was the organism recovered from leaves dried as long as 2 months.

Exposure to sunlight for 15 minutes killed 100 per cent of the organisms.

RELATION TO HOST TISSUES

Microscopic examination of the lesions shows that the organism attacks the parenchyma. The intercellular spaces and the cavities formed by disintegration of cell walls become packed with the bacteria.

For a study of the early stages, material was secured from plants inoculated by spraying with pure cultures of the bacteria suspended in water. As soon as infection was evident the tissues were collected and fixed. Stained sections of this material show that the bacteria enter by way of the stomata (Pl. 2, B and E). In the earliest stages the bacteria are found only in the stomatal chamber, from which they spread into and fill adjoining intercellular spaces. That some chemical change occurs at this stage in the cell walls adjoining the masses of bacteria is evidenced by their increased affinity for stains. Whether they are later dissolved or only crushed by pressure and so changed chemically as to be vague and doubtful is not yet determined, but cavities occur and become entirely filled with the parasite. In lesions of considerable size no definite cell-wall structure remains in the central space. In some large cavities the bacteria are arranged in layers (Pl. 2, A).

COMPARISONS WITH SOME SIMILAR BACTERIAL DISEASES

The translucency of the lesions and the yellow color of the bacterial growth suggested that this leaf spot might be identical with some previously described bacterial disease on monocotyledons. However, careful comparisons show that *Bacterium gummisudans* has characters that separate it from the several organisms that it, to a certain degree, resembles. *Bacterium translucens* Jones, Johnson, and Reddy[6] and *Bacterium translucens* var. *undulosum* Smith, Jones and Reddy,[6,8] causing disease on barley and wheat, respectively, are unlike *Bacterium gummisudans* in cultural characters in several media and in the consistency of the bacterial growth. Moreover, *Bacterium gummisudans* does not infect grains. Four varieties of barley were used in the inoculation experiments. Several tests were made, but no infections resulted. Control inoculations on gladioli gave typical infections. *Aplanobacter rathayi* EFS.[7,8] and *Aplanobacter agropyri* O'Gara[9] (on western wheat grass) are nonmotile organisms, while *Bacterium gummisudans* is a motile organism.

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[6] JONES, L. R., JOHNSON, A. G., and REDDY, C. S. BACTERIAL-BLIGHT OF BARLEY. *In* Jour. Agr. Research, v. 11, p. 625–644, 2 fig., pl. B (col.), 47–49. 1917. Literature cited, p. 643.

[8] SMITH, Erwin F., JONES, L. R., and REDDY, C. S. THE BLACK CHAFF OF WHEAT. *In* Science, n. s., v. 50, p. 48. 1919.

[7] SMITH, Erwin F. BACTERIA IN RELATION TO PLANT DISEASES. v. 3, p. 155–160. Washington, D. C. 1914. (Carnegie Inst. Wash. Pub. 27.)

[8] —— AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. fig. 12–22. Philadelphia and London. 1920.

[9] O'GARA, P. J. A BACTERIAL DISEASE OF WESTERN WHEAT GRASS, AGROPYRON SMITHII. *In* Phytopathology, v. 6, p. 341–350, pl. 9–13. 1916.

TECHNICAL DESCRIPTION

Bacterium gummisudans, n. sp.

A short rod, rounded at the ends, individual rods 1 to 2.8μ by 0.6 to 0.8μ; motile by means of one polar flagellum 3 to 9μ long, capsules present, no spores; aerobic. A yellow, viscid growth is formed on culture media; nitrates are not reduced; casein in milk is digested; gelatin is liquefied; acid is formed from dextrose and saccharose; ammonia and hydrogen sulphid are produced; optimum temperature about 30° C., maximum 36°, minimum below 2°; thermal death point near 50°. Sensitive to sodium chlorid and to acids; readily killed by drying and by exposure to sunlight. Gram-negative; not acid-fast. Group number 211.2322523.

Pathogenic on leaves of gladioli forming more or less angular, translucent spots.

Type specimens have been deposited in the herbarium of the Bureau of Plant Industry, United States Department of Agriculture.

SUMMARY

The bacterial disease described in this paper is capable of causing serious injury to the leaves of the gladiolus and consequently it interferes with the development of the corms.

The lesions are more or less angular, translucent spots. From the infected tissues there is a rather copious and viscid exudate which when dry forms a thin, brittle layer, or small drops over the surface. The leaves are often coated with soil particles which have become embedded in the exudate.

The bacteria are very abundant in the infected areas and pure cultures are easily isolated. On culture media this bacterium produces a yellow, viscid growth. It is motile, having one flagellum; capsules are present; no spores. It is Gram-negative and not acid-fast. The group number is 211.2322523.

Numerous inoculations resulting in successful infections have proved that the bacterium isolated from the leaves is the cause of the disease.

The organisms gain entrance to the tissues by way of the stomata. They invade the parenchyma and fill the intercellular spaces and cavities resulting from destruction of cell walls.

PLATE 1

Bacterium gummisudans on gladiolus

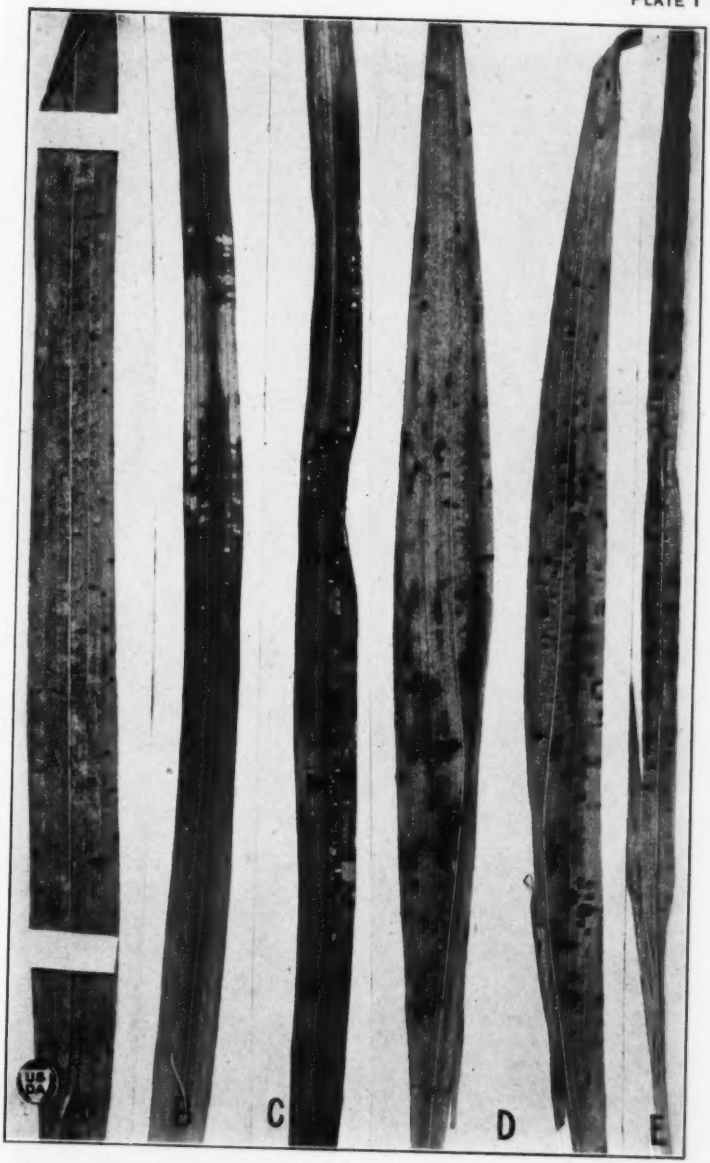
A.—Early stage of the bacterial blight, showing the water-soaked, horizontal lesions. Natural infection. ×1.

B.—Artificial inoculation 18 days after inoculation. Photographed by transmitted light. ×1.

C.—Artificial inoculation showing the dry exudate on the lesions. ×1. A, B, and C are leaves from "seedling" plants.

D.—Leaves from blooming plants (var. Schwaben) showing general appearance in the field. Natural infections. ×1/2.

E.—Leaf from seedling plant (var. Schwaben). Natural infection. ×1.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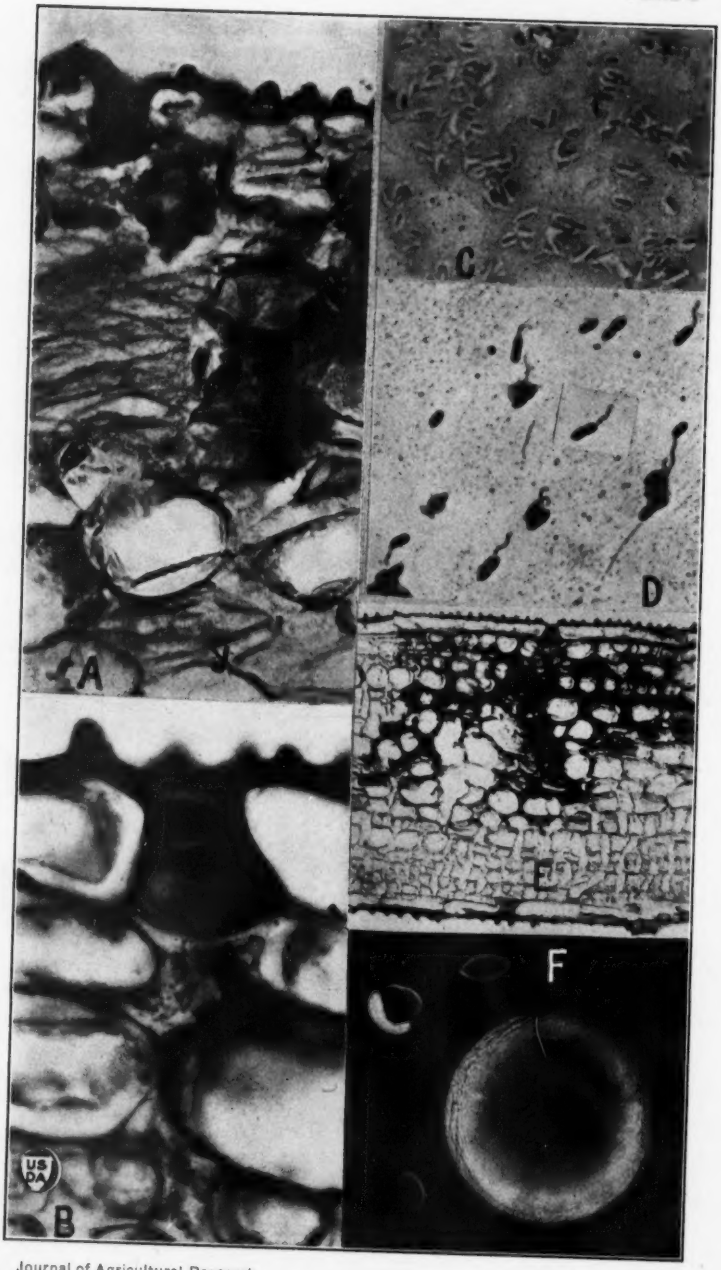
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PLATE 2

Bacterium gummisudans on gladiolus

A.—Lesions showing bacteria arranged in layers in the central cavity and also in the cells *x* and *y* where a part, at least, of the cell wall still exists.

B.—Early stage of stomatal infection. Only a few bacteria are in the stomatal chamber and the adjoining intercellular spaces. × about 1,000.

C.—Capsules. Bacteria from a 4-day beef agar culture. ×2,000.

D.—Flagella. Bacteria from 2-day-old beef agar culture. Casares-Gil's stain. Bacteria are viscid and it is difficult to get an even distribution of single rods. ×2,500.

E.—Later stage than B. Bacteria in great numbers have penetrated widely into the intercellular spaces but the cell walls are as yet only slightly affected.

F.—Surface and buried colonies on beef agar plate (3 weeks old). The surface colony shows the characteristic striations. ×5.